Vet. Med.
RECEIVED
JAN 13
VET. MED. LIBRARY

The Veterinary Record

PATRON: HER MAJESTY THE QUEEN

OHIO STATE UNIVERSITY
JAN 13 1960
LIBRARY

Vol. 71 No. 43 Issue No. 4255 901-920

BIOGLAN

natural VITAMIN-E

DO YOU KNOW that, quite apart from its value in certain diseases and as reported in the Veterinary Press, *natural* vitamin E

IMPROVES :– **temperament reactions, staying power, condition**

REDUCES :– **heart strain, incidence of cramp, nervousness**

and is being employed with great advantage by many leading breeders and owners of sporting and exhibition dogs.

✱ Supplied in tablets, it is inexpensive, completely safe and easy to administer, but it *must* be a stable derivative of *natural* alpha tocopherol which combines uniform strength and high concentration with complete absorption.

Full Details Available from:—

BIOGLAN LABORATORIES LTD

(VETERINARY DIVISION), HERTFORD, HERTS.

Our ORIGINAL

CHLORAMPHENICOL AEROSOL

has proved its efficiency

It ensures a quick and deep penetration of the minute particles, and it is clean and easy to use. The inclusion of gentian violet acts as a marker.

Each unit of CHLORAMPHENICOL AEROSOL 10% contains sufficient material for 40 to 50 feet.

Available in cases of:

6 × 114 gramme units *and*
12 × 114 gramme units

SOMERSET PHARMACEUTICALS LTD
WELLINGTON SOMERSET - *Phone:* Wellington Som. 2244

The
VETERINARY RECORD

Founded by William Hunting, F.R.C.V.S., in 1888
7, MANSFIELD STREET, PORTLAND PLACE, LONDON, W.1. MUSEUM 6541

No. 43 DECEMBER 12th, 1959 Vol. 71

Registered for Transmission as a Newspaper *Price 2s. post free*

Bronchography in the Dog

BY

S. W. DOUGLAS and L. W. HALL

Department of Veterinary Clinical Studies, University of Cambridge

SUMMARY.—Methods of bronchography which have been employed in the dog are discussed. Results obtained by the use of similar methods are recorded and the evolution of a more satisfactory technique is described. The technique evolved has been used in healthy experimental animals and in dogs affected with chronic pulmonary disorders such as bronchiectasis.

Introduction

IN man bronchography has become a routine procedure in the investigation of various types of respiratory disease, but in veterinary medicine it appears to be a technique about which little is known. Bishop, Medway and Archibald (1955) have described a technique in which a radio-opaque medium is injected through a polythene tube which has been introduced into the bronchial tree by passing it down an endotracheal tube, while Dyce (1955) has described a similar method without the use of an endotracheal tube. In both of these methods, while the radio-opaque material is being introduced into the bronchial tree, the posture of the dog is altered to ensure filling of the bronchi to the various lobes of the lung, and both are said to produce satisfactory results. The method which is described below has certain possible advantages over the two foregoing techniques and has never, in an admittedly limited number of cases, failed to produce X-ray films of diagnostic value.

Evolution of the Method

Early in the present studies radio-opaque materials were introduced into the main bronchi to the various lobes of the lung by means of a polythene tube, which was passed into the individual bronchi through a bronchoscope under direct vision. Anaesthesia was induced with thiopentone after premedication with atropine, and the bronchial tree was thoroughly sprayed with a 4 per cent. solution of lignocaine hydrochloride prior to bronchoscopy. Satisfactory X-ray films were not obtained because the radio-opaque material was not distributed evenly and the alveoli were frequently filled. However, the observation of Dyce (1955) that an aqueous preparation of propyliodone* is the most satisfactory contrast medium was confirmed. It was also found to be necessary to examine each lung separately.

As a further development, attempts were made to inject this aqueous medium through a polythene tube introduced into the left or right main bronchus *via* an endotracheal tube. The posture of the animal was adjusted during the injection to aid the spread of the radio-opaque medium throughout the bronchial tree. Anaesthesia was again produced with thiopentone after atropine premedication and not infrequently the animal coughed as the propyliodone ran into the bronchus. Contrary to the findings of Bishop *et al.* (1955) coughing was found to result in dispersion of the medium into the alveoli with consequent loss of pattern. In later cases this troublesome complication was overcome by paralysing the anaesthetised animal with suxamethonium and positive pressure respiration was carried out before and after the introduction of the radio-opaque substance. Good X-ray films were not always obtained, but gross bronchiectasis was satisfactorily demonstrated by this technique.

The next development in the evolution of the method was the adoption of the "drowning technique" (Jacoby & Keats, 1938; Temple & Gray, 1950) as used for bronchography in children (Parkhouse, 1957). This technique has been modified for

* Dionosil Aqueous; Glaxo.

use in dogs and has proved to be both simple and safe while yielding very satisfactory X-ray films.

Principles of the Method

1. *Preparation*

Clinical cases may require pre-radiological preparation with antibiotics and expectorants to eliminate tenacious sputum from the bronchial tree, for if sputum is present at the time of examination it may plug the bronchi to the diseased segments of the lung and prevent the spread of the radio-opaque medium.

2. *Premedication*

Premedication is with atropine alone, because it is essential that the cough reflex should be active at the end of the procedure.

3. *Anaesthesia*

Anaesthesia is induced with a very small dose of thiopentone sodium given by intravenous injection, then deepened with ethyl chloride or divinyl ether followed by diethyl ether. The aim is to produce third plane diethyl ether anaesthesia as rapidly as possible for, if this is achieved, the high initial concentration of ether in the cerebral circulation will give a long enough period of anaesthesia for taking the X-ray films, and the dog will awaken quickly as a result of redistribution of the ether in the body. It is important to note that this awakening does not depend on the excretion of ether through a layer of radio-opaque material, and thus the vicious circle of slow induction and slow recovery is avoided. It has been found that the rapid induction of deep anaesthesia is most easily achieved when the ether is administered by the open drop method and usually takes about 6 minutes from the start of the thiopentone injection.

4. *Radiography*

The administration of the anaesthetic is stopped when the third plane of the third stage has been reached and all ether is removed from the vicinity of the X-ray apparatus. The dog is placed in lateral recumbency, the lung which is to be examined being on the underside, the head is raised off the table and the mouth opened. The radio-opaque medium is poured rapidly over the back of the tongue into the pharynx, the mouth is closed and any residual material wiped from the lips with a gauze swab. After the introduction of the material into the pharynx the dog is held in position for approximately 2 minutes, the object being to fill all segments of the lungs by means of the dog's spontaneous respiratory movements. Thus the nearest possible approach is obtained to the dog's natural breathing condition and from the appearance of the films it is possible to form some idea of the functional as well as the anatomical extent of the disease. A lateral film is taken about 3 minutes after the inhalation of the material is heard to begin. The subsequent procedure depends on whether the left or the right lung is being examined.

To demonstrate the bronchial tree of the left lung the dog is rolled over immediately after the lateral film has been taken and a ventro-dorsal or dorso-ventral film is taken as quickly as possible.

In the case of the right lung, however, it has been found that the posture of the dog has to be adjusted if the bronchus to the intermediate lobe is to be outlined. Immediately after the exposure of the lateral film the dog is turned into the prone position; its body inclined head up at an angle of about 20 to 30 degrees and slightly rotated so that the right chest wall is higher than the left. This position is maintained for about 1 minute after which a dorso-ventral film is taken. The animal is then turned so that it is lying on its right side and a second lateral film exposed.

All radiographs are taken on full inspiration using the shortest possible exposure time (preferably not more than 0.025 seconds).

As soon as the necessary films have been obtained the dog is positioned so that the lung which has been filled is uppermost and the chest percussed to stimulate coughing. Coughing soon occurs and although it is desirable that both oxygen and suction should be available, cyanosis has not been seen. The coughed up material is usually swallowed and if further radiographs are taken about 20 minutes after the administration of the propyliodone, it will be seen that much of the material has been cleared from the lungs and is visible in the oesophagus and stomach. Removal of the contrast medium may be assisted by the introduction of a catheter into the trachea and the application of suction through this catheter while it is moved about to promote coughing.

Obviously the procedure cannot be repeated for the examination of the second lung until all material has been cleared from the first lung. Experience has shown that the contrast agent could no longer be recognised in radiographs taken after 48 hours from the time of introduction.

Quantity of Radio-opaque Material Used

It was found that the quantity of contrast medium required to produce well-filled bronchograms is related not to the size of the dog but rather to the size of the largest endotracheal tube which can be introduced into the trachea. For a dog which can accommodate a No. 3 Magill tube about 5 ml. of medium is required, while if a No. 10 Magill tube can be passed about 20 ml. should be used. For Greyhounds in which a V12 endotracheal tube can be employed, good well-filled bronchograms have been obtained by the use of between 25 and 30 ml. of the propyliodone for each lung.

Interpretation of Bronchograms

(a) *Normal Dogs*

Most of the work detailed above has been carried out on the "normal" experimental animal. Experience has shown that, provided the above technique is carried out carefully, the resulting radiograph will provide a good clear-cut outline of the primary, secondary and tertiary bronchi of each lobe of lung under examination (Plates I and II). An apparent hold-up in the passage of contrast media

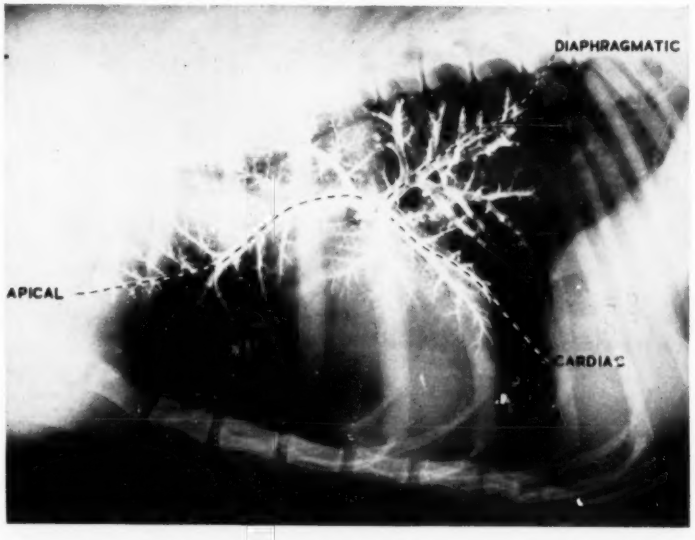

PLATE I. -Normal lateral bronchogram of left lung of a Greyhound. The main bronchus of each lobe is identified.

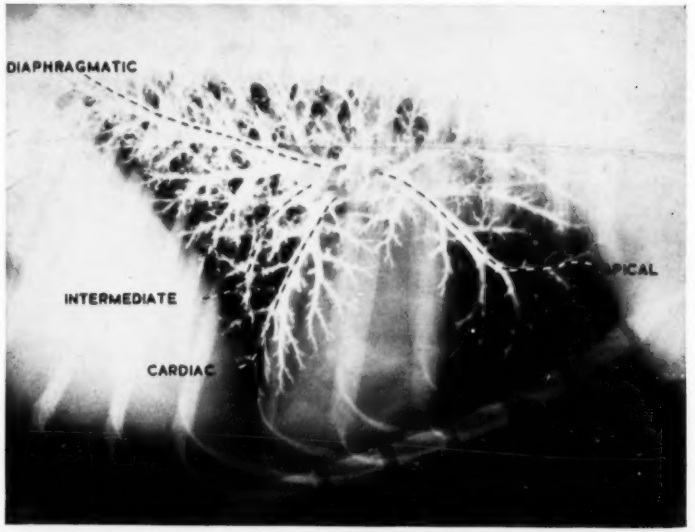

PLATE II.—Normal lateral bronchogram of right lung of a Mongrel.

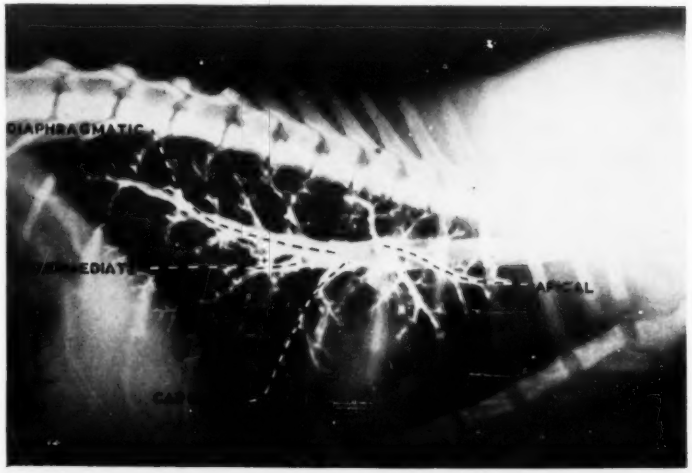

PLATE III.—Normal lateral bronchogram of right lung of a cat.

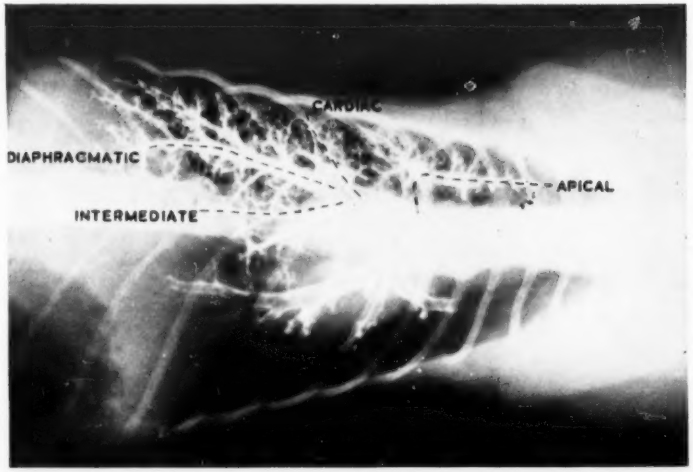

PLATE IV.—Normal ventro-dorsal bronchogram of right lung of the cat shown in Plate III. Note that some overflow of contrast medium into the left bronchial tree occurred.

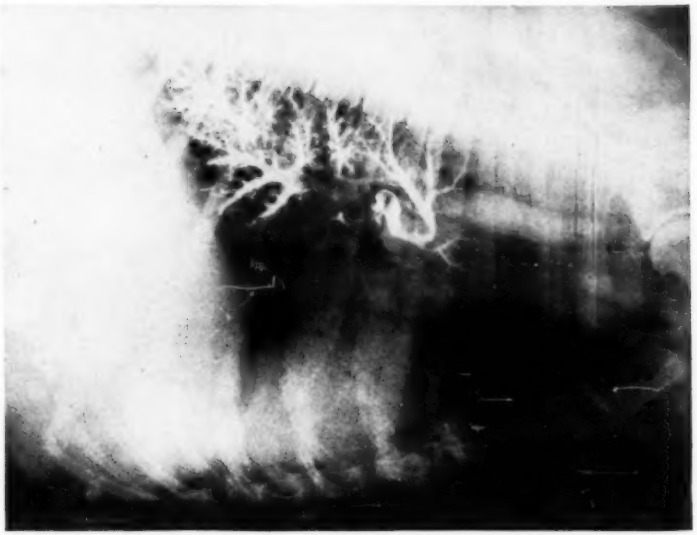

PLATE V.—Lateral bronchogram of right lung of a Collie. The contrast medium failed to fill cardiac and intermediate bronchi but demonstrated displacement of the apical lobe.

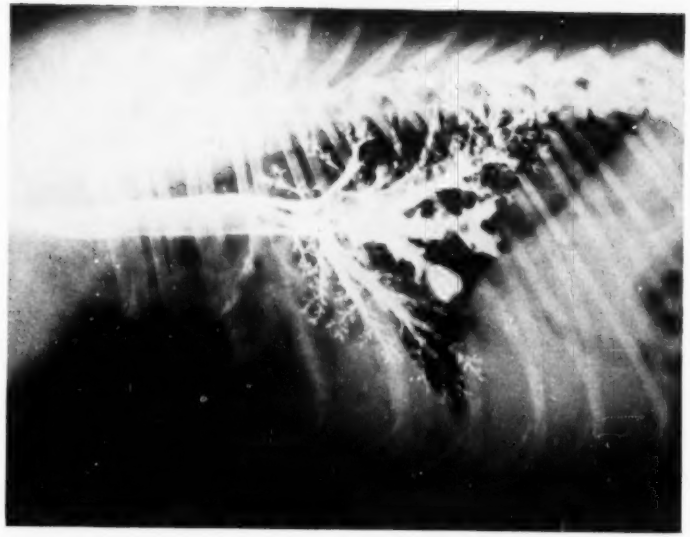

PLATE VI.—Lateral bronchogram of left lung of a Pomeranian. There was failure to outline apical bronchi but extensive bronchiectasis of diaphragmatic lobe was shown.

PLATE VII.—Lateral bronchogram of right lung of the dog shown in Plate VI. Gross and extensive bronchiectasis demonstrated.

along the larger bronchi may be due to the fact that insufficient time has been allowed for it to spread to the terminal portion of the bronchus. This can be confirmed by showing further spread in a subsequent radiograph, but the question of timing of the radiographs is somewhat critical, since if they are delayed for too long, the animal may be awakening from anaesthesia and moving, or the contrast medium may spread into the alveoli and cause blurring of the radiograph. Another cause of poor filling of the bronchial tree is due solely to the introduction of an insufficient quantity of contrast medium.

Apparent arrest of the contrast medium in the finer bronchi does occur in the normal dog; this is presumably due to the presence of mucus, and it does not appear to be of significance.

While the radiographs produced in each of the two planes have been completely satisfactory for diagnostic purposes it has been found that those taken in the dorso-ventral plane do not reproduce well photographically (and are, therefore, not shown in this article) because the greater density of the heart and vertebral column allow of little contrast with the density of the medium employed.

It was possible, however, to obtain good contrast in the case of a cat in which the same technique was used for bronchography. Bronchograms taken in both planes of the right lung of this animal are shown, since they do effectively demonstrate the position of the intermediate lobe bronchus (Plates III and IV).

(b) *Clinical Cases*

In conjunction with the above investigation of the normal animal, the method has also been applied to suitable clinical cases. These cases have been carefully selected and the procedure has been confined to dogs with a history of a chronic cough in which a straight film of the chest suggested the presence of lung changes and bronchoscopy revealed no obvious cause for these changes, *e.g.* the presence of parasitic nodules.

During a period of 8 months bronchography was performed on 6 dogs and in 4 the findings were considered to be of pathological significance. Brief histories of these are summarised as follows :—

Case 1. 3-year-old Corgi bitch. Presented for a chronic cough of over a year's duration. Bronchography carried out by the earlier methods described above revealed severe bronchiectasis which was confined to the intermediate and diaphragmatic lobes of the right lung. Resection of these lobes was followed by temporary relief but the cough returned and persisted. Bronchography was repeated 18 months after the previous examination and showed that extensive bronchiectasis had developed in the left lung.

Case 2. 4-year-old Boxer dog. Seen on account of a progressive cough noticed for 5 weeks. Bronchography indicated slight but definite bronchiectasis of both diaphragmatic lobes and of the intermediate lobes.

Case 3. 12-year-old Collie bitch. History of a cough of approximately a year's duration. Bronchography suggested that there was displacement of the apical lobe of the right lung (Plate V). This was confirmed at *post-mortem* examination.

Case 4. 6-year-old Pomeranian dog. Attacks of coughing, which were becoming more severe, had been noticed for 6 months. Bronchography revealed extensive bronchiectasis of both lungs (Plates VI and VII). This diagnosis was confirmed at *post-mortem* examination.

PLATE VII.—Lateral bronchogram of right lung of the dog shown in Plate VI. Gross and extensive bronchiectasis demonstrated.

It has already become apparent that bronchiectasis occurs more frequently in the dog than had been previously realised. It is hoped, therefore, to publish a further paper dealing with the clinical aspects of the condition when a larger number of cases have been examined.

Acknowledgments.—We wish to acknowledge the valuable assistance of Miss J. Minns and Mr. H. D. Williamson who were responsible for the photographic and radiographic work involved in the production of this paper. We have been dependent on local veterinary surgeons for the opportunity to examine the clinical cases recorded above, and we gratefully acknowledge their willing co-operation. Finally, we would thank Messrs. Glaxo for providing a generous supply of propyliodone for the purpose of this investigation.

References

BISHOP, E. J., MEDWAY, W., & ARCHIBALD, J. (1955). *N. Amer. Vet.* **36.** 477.
DYCE, K. M. (1955). *Brit. Vet. J.* **111.** 319.
JACOBY, N. M., & KEATS, G. (1938). *Lancet.* **ii.** 191.
PARKHOUSE, J. (1957). *Brit. J. Anaesth.* **29.** 447.
TEMPLE, L. J., & GRAY, T. C. (1950). *Lancet.* **ii.** 116.

IN PARLIAMENT

Pigs

Mr. Hendry (December 8th) asked the Secretary of State for Scotland if he will state the number of pigs slaughtered in Scotland in the period of twelve months to the latest convenient date for bacon and pork, respectively; and how these compare proportionately with the corresponding figures for England and Wales.

Mr. Maclay: During the twelve months to the end of September, 1959, approximately 300,000 pigs were put into cure by bacon curers in Scotland, and a further 250,000 pigs were used for other purposes. The corresponding figures for England and Wales during the same period were 2·5 million pigs put into cure and 6·7 million for other purposes.

Mr. Hendry: While thanking my right hon. Friend for that reply, may I ask whether, since the price of pork pigs is generally on average higher than the price of bacon pigs, Scottish rearers are not at a considerable disadvantage compared with rearers in England? May I ask whether at the next Price Review he will press for separate treatment for Scotland?

Mr. Maclay: Not all pigs sold to bacon curers are used for bacon. The rest of the supplementary question is rather complicated. I should like to consider it and write to my hon. Friend about it.

Poisonous Substances (Advisory Committee's Recommendations)

Mr. Eden (December 8th) asked the Minister of Agriculture, Fisheries and Food if he has completed his consultations about the recommendations of the Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage; and if he will make a statement.

Mr. John Hare: These consultations are not yet completed, but as soon as they are I will make a statement.

Active Immunisation of Sheep and Horses Against Tetanus with Aluminium-Hydroxide-Adsorbed Toxoid

BY

K. S. CHODNIK, A. R. A. WATSON, and J. R. HEPPLE

Glaxo Laboratories Ltd., Greenford, Middlesex

A REVIEW of veterinary publications during the last 40 years shows that, despite all the efforts made, successful treatment of tetanus in animals has not been achieved and mortality among treated cases remains high. There is every indication that, for the foreseeable future, the only real approach to the tetanus problem will still be the prophylactic one.

The action of hyperimmune serum is effectively preventive, but unfortunately lasts only about 2 weeks. To attain full passive protection after injury it is necessary to repeat injections of serum, with all the consequent dangers of multiple serum therapy.

In contrast, extensive investigations in recent years on animals and on man have demonstrated beyond doubt the overwhelming superiority of active immunisation. Those of the last 2 decades first of all cleared up conclusively some persisting confusion and misinterpretations on active immunity to tetanus and, second, proved convincingly the efficacy of active immunisation.

Ramon's discovery (1924) that toxin detoxified by the action of formol is capable of stimulating specific antibody formation opened the way to wide practical application. Injection of such detoxified toxin (in French "anatoxin" and "toxoid" in English) brings about the appearance in the circulating blood of antitoxin similar to that appearing there after the injection of hyperimmune serum.

The original technique of actively immunising animals introduced by Ramon (1931) has since been greatly improved, first by purifying the crude formol toxoid and second by adsorption of the purified products on aluminium compounds, of which aluminium hydroxide was shown to give the most potent adsorbates (Levine *et al.*, 1955).

Since a prophylactic injection of 1,500 antitoxin units (A.U.) in hyperimmune serum brings the level of blood antitoxin in man to 0.1 to 0.25 A.U. per ml. serum, it was assumed that such a level of antitoxin is probably also required after active immunisation. In the absence of more precise information, the general opinion persisted that animals actively immunised remain resistant to the disease only as long as sufficient free antitoxin is detectable in their blood. There were, however, considerable differences of opinion on the level of antitoxin considered significant for immunity. This is exemplified in figures quoted by different workers, for example:—

(1) 0.1 A.U. McComb and Trafton (1950).
(2) 0.01 A.U. Sneath *et al.* (1937).
(3) 0.0025 A.U. Ramon (1937).

Realising that the most convincing criterion of actively induced immunity should be the power to withstand infection with *Clostridium tetani* spores, Sneath *et al.* (1937) carried out an experiment on guinea-pigs by the method of direct challenge. Since actively immunised animals with less than 0.01 units of antitoxin per ml. of serum also survived in this trial, he suggested that the amount of antitoxin might not be the sole factor involved in protection against a lethal dose of spores. This suggestion has been fully substantiated in a series of exhaustive studies by Wolters and Dehmel (1940, 1942-3), Wolters and Fischoeder (1953), Saxinger and Heinig (1951), and Becker and Pilz (1955).

In numerous experiments on small laboratory animals, as well as on horses, sheep, and man, methods of direct challenge have been extensively used for the evaluation of active immunity.

The results of these studies are particularly important for the proper understanding and assessment of active immunity. They proved that true immunity can be defined only in terms of resistance to infection and not, as previously thought, in terms of antitoxin units. By exposing the actively immunised animals to direct challenge, either as free toxin or as suspensions of spores, it was found that all animals receiving 2 doses of potent adsorbed toxoid attained, without exception, a high degree of protection and withstood a massive challenge, whereas the unvaccinated controls developed tetanus. When the amount of detectable antitoxin at various times was compared with the power to resist infection, it became evident that they were not related directly to each other.

On injection of toxoid the amount of antitoxin reaches its highest value soon after the injection and then begins to fall steadily in each immunised animal. However, when these animals were exposed to direct challenge at various times after immunisation, they continued to remain resistant to large challenging doses even when their antitoxin level had fallen below that previously accepted as indicating immunity. The fact that every challenge, even years after vaccination, is followed by the re-appearance of antitoxin is fairly convincing evidence that the original antigenic stimulus must primarily have affected cellular elements. Such cells, once adequately stimulated, become so modified or trained that even years later, on exposure to renewed stimuli either as toxin, in natural infection, or as toxoid, in booster doses, they respond rapidly with a new release of antitoxin.

In certain circumstances, however, as for instance before surgical operation, the use of 2 doses of vaccine may be impracticable. Saxinger and Heinig (1951) undertook separate experiments to define the earliest time after the injection of aluminium

hydroxide adsorbate at which animals will successfully resist direct challenge. In their experiments, horses and sheep were injected with a single dose of toxoid and were challenged within the next 20 days. Horses receiving a single dose of toxoid showed a high degree of protection 12 days, and sheep 10 days, after the injection. Fifty-two horses so protected withstood a challenge of 3 MLD of toxin, whereas all 16 controls succumbed to a dose of 2 MLD. This important finding indicated another practical value of active immunisation. Ramon and Lafaille (quoted by Wolters and Dehmel) and Wolters and Dehmel (1942-3) demonstrated that simultaneous injection of hyperimmune serum (antitoxin) and toxoid are compatible and act independently without interference. Since it is known that serum therapy is effective up to 12 days from the day of injection, and that a single injection of aluminium hydroxide adsorbed toxoid is already effective 12 days after injection, there is only one logical course of action after injury of animals not previously immunised, namely to apply a single dose of serum along with the toxoid, which can if desired be given again to secure lasting active immunity. This was fully confirmed on sheep and guinea-pigs by Heinig (1954).

On the efficacy of active immunisation, we may quote Becker and Pilz (1955) to the effect that in the Dresden Serum Institute no case of tetanus was encountered among horses during the previous 20 years when active immunisation had been generally adopted. But the real value of toxoid immunisation cannot be fully appreciated without reference to Ramon's review of tetanus vaccination in the armies during the World War II (1949). In the British Army, where mass vaccination was practised, among 103,000 soldiers injured on the Western Front only 6 cases of tetanus were reported; at the same time there were 25 cases among a small number (a few thousands only) of unvaccinated injured prisoners. Even more convincing were the results obtained in the American Army. Reports up to 1947 showed that on all fronts only 12 cases of tetanus were encountered, all of them before vaccination became compulsory.

Our Own Investigations

Introduction

Clinical reports of recent years indicate that tetanus among young lambs is not uncommon and that the incidence would justify taking prophylactic measures. Apart from neonatal tetanus, castration by means of rubber rings is claimed from time to time to be the second commonest predisposing factor. Our investigations were, therefore, undertaken initially to evaluate purified tetanus toxoid adsorbed on aluminium hydroxide as a product suitable for immunising sheep. Though it was assumed that separate immunisation of sheep against tetanus would be of doubtful practical value except in some circumstances, it was decided to explore the possibility of incorporating tetanus with pulp kidney toxoid into a combined tetanus/pulpy kidney vaccine on the lines of the tetanus/diphtheria vaccine used for human prophylaxis. As we met a complete absence of any local reactions in sheep we decided to test it on horses as well.

Material and Methods

For immunisation the tetanus toxoid used was prepared in Glaxo Laboratories and purified by ammonium sulphate precipitation and dialysis. Such purified toxoid was adsorbed on to preformed aluminium hydroxide, separately or along with purified pulpy kidney toxoid. Both preparations were first tested on small laboratory animals for local and general reactions, compatibility of the 2 antigens, and antibody response.

Antibody response was assayed by the amount of antitoxin found after the injections. Titration of the tetanus antitoxin, expressed in international units, was done by antitoxin-toxin neutralisation test and by assays on mice against standard toxin-antitoxin mixtures. For this work a precision of 0.01 antitoxin units per ml. of serum was accepted as satisfactory.

All the animals, small and large, were individually tested for the presence of detectable antitoxin before their use in the experiment. Only animals showing no detectable antitoxin were used.

As a basis for immunisation 2 doses of toxoid were adopted. The second dose was given 3 to 4 weeks after the first, mostly after 4 weeks. A volume of 2 ml. of inoculum was chosen for both sheep and horses. The optimum concentration of toxoid was tested on each species separately. The animals were bled for antitoxin titrations, generally before the second injection, always 3 weeks after the second injection, and subsequently at the times specified.

Experimental

When the initial trials on small animals proved satisfactory, and the compatibility of tetanus and pulpy kidney toxoids used concurrently was assured, trials were undertaken on sheep.

Trials in Sheep

Two groups of sheep were used. Group I comprising 14 young lambs (12 ewes and 2 rams) were kept entirely indoors after birth. Ten ewes and one ram of this group received 2 injections of combined tetanus/pulpy kidney toxoid at 1½ to 2 months of age. The remaining 2 ewes and one ram, when 4 months old, received 2 injections of tetanus toxoid alone at the same concentration. These animals were bled for antitoxin titration before the second injection, 3 weeks after the second injection, and subsequently at monthly intervals. Ewes of this group were mated and at 10 months of age while pregnant received one booster dose of the same toxoid as for the initial immunisation; 3 weeks later the antitoxin titres were determined again. The results of tetanus antitoxin titrations on lambs immunised with mixed tetanus/pulpy kidney toxoids are summarised in Table I.

Group II comprised 12 old cross-bred ewes that had previously been used for toxicity tests on pulpy kidney vaccines and consequently were selected in this work only for the evaluation of tetanus toxoid adsorbate. Five ewes of this group were given 2 smaller doses of toxoid at the time of tupping. Their

TABLE I
TETANUS ANTITOXIN TITRATIONS ON SHEEP IMMUNISED WITH THE COMBINED TETANUS/PULPY KIDNEY TOXOIDS

Sub-group	Number of animal	Antitoxin units per ml. of serum (A.U. 1950)									
		Pre immunisation	Pre second injection	After second injection							Three weeks after booster dose
				3 weeks	2 months	3 months	5 months	6 months	7 months	8 months	
A	B1	<0·01	0·1	9·0	3·0	1·0–2·0	0·2 –0·4	0·2 –0·4	0·1–0·2	Booster	40–60
	B3	<0·01	1·6	18·0	4·0	2·0–3·0	0·4 –1·0	0·4 –1·0	0·4	,,	20–40
	B5	<0·01	0·4	0·8	0·4	0·6–0·8	0·2 –0·4	0·2 –0·4	0·1–0·2	,,	10
	B6	<0·01	0·2	5·4	1·5	1·0–2·0	0·4 –1·0	0·4 –1·0	0·2–0·4	,,	20–40
	B8	<0·01	0·05	2·2	0·8	0·2–0·4	0·04–0·1	0·04–0·1	0·04	,,	10–20
	B24	<0·01	0·1	1·0	0·4–1·0	0·2–0·4			Discontinued		
B	B9	<0·01	0·2	13·5	6·4	2·0–3·0	0·4 –1·0	0·4	0·2–0·4	Booster	4–10
	B12	<0·01	0·4	9·0	6·4	3·0–4·0	1·0	0·4 –1·0	0·4–1·0	,,	10–20
	B13	<0·01	0·4	40·0	10·0	3·0–4·0	2·0 –4·0	1·0 –2·0	1·0–2·0	,,	60
	B15	<0·01	0·8	13·5	12·0	3·0–4·0	1·0 –2·0	0·4 –1·0	0·4–1·0	,,	20–40
	B19	<0·01	0·3	11·25	3·0	1·0–2·0	0·4 –1·0	0·4 –1·0	0·2–0·4	,,	4–10

antitoxin levels were determined 3 weeks, 2 months, and 3 months after the second dose of toxoid. To study the transfer of maternal immunity to offspring, a single booster dose of tetanus toxoid was given 4 months after immunisation, and the antibody response was again measured. Eight remaining ewes of Group II, injected in late pregnancy, received a larger dose of toxoid, and their antitoxin levels were determined before the second dose and also 3 weeks after the second dose. Thereafter these animals, together with all the ewes used in this experiment, were assigned for a separate study on the transfer of immunity from mother to offspring. The results of these studies we plan to submit for publication separately. Antitoxin titrations on ewes and lambs immunised with tetanus adsorbate alone are given in Table II. As seen from Tables I and II, the antigenic responses expressed in antitoxin production after injection of purified tetanus toxoid were remarkably constant. The generally accepted individual differences in the amounts of antitoxin are demonstrated in both Tables. Other factors influencing the degree of antigenic response are also shown in these Tables. Among these the age of the vaccinated animals appears of importance. As seen from Table I, younger animals appear to show a marked advantage over the older, as expressed in the higher amount and longer persistence of the antitoxin in their blood.

It is clearly shown in Table II that the older animals are by no means unsuitable for immunisation; despite their overall lower antitoxin levels, and also the quicker fall in their blood antitoxin levels, they responded as well to a single booster dose of toxoid as did the younger animals. The influence of toxoid concentration on antigenic response is also shown; the lower figures for antitoxin in the A subgroups correlate with the smaller and the higher figures for the B subgroups with the larger amounts of toxoid injected.

TABLE II
TETANUS ANTITOXIN TITRATIONS IN SHEEP IMMUNISED WITH TETANUS TOXOID ONLY

Sub-group	Number of animal	Antitoxin units per ml. of serum (A.U. 1950)						
		Pre immunisation	Pre second injection	After second dose			Booster dose 4–5 months after second dose	Titre 5 weeks after booster dose
				3 weeks	2 months	3 months		
A	B26	<0·01	—	0·6– 1·0	0·4–1·0	0·4 –1·0	+	10·0–20·0
	B27	<0·01	—	0·8– 2·0	0·8–2·0	0·8 –1·0	+	10·0–20·0
	B25	<0·01	—	1·0– 2·0	1·0–2·0	0·4 –1·0	Not given	—
	A7	<0·01	—	0·6– 1·2	0·4–1·0	0·1 –0·2	+	2·0– 4·0
	A10	<0·01	—	0·1– 0·2	0·1–0·2	0·04–0·1	+	4·0– 8·0
	A13	<0·01	—	0·2	0·1	0·04–0·1	+	10·0–15·0
	A14	<0·01		0·4– 0·8	0·4–0·8	0·4 –0·8	+	4·0– 8·0
B	A3	<0·01	0·2	1·0– 2·0	—	0·4 –1·0	—	—
	A5	<0·01	0·2	0·4– 1·0	—	0·02–0·04	—	—
	A6	<0·01	2·0	20·0	—	0·4 –1·0	—	—
	A8	<0·01	0·1	0·4– 1·0	—	0·4 –1·0	—	—
	A11	<0·01	0·2–0·5	1·0– 2·0	—	1·0 –2·0	—	—
	A12	<0·01	0·2	1·0– 2·0	—	0·4 –1·0	—	—
	A18	<0·01	1·0–2·0	6·0–10·0	—	1·0 –2·0	—	—
	A19	<0·01	0·2–0·5	1·0– 2·0	—	0·2 –0·4	—	—

Titrations for the ε antitoxin of *Cl. welchii* type D in lambs immunised with mixed tetanus/pulpy kidney toxoid carried out on the same blood samples as those assayed for tetanus antitoxin showed that the lambs without exception responded with a marked antitoxin rise, in every animal well above

the accepted minimum of 0.2 antitoxin units per ml. of serum, with an average of 3.5 units. All the lambs also showed an excellent response to a booster dose, attaining an average of 5.4 to 12.4 antitoxin units per ml. serum. The results of antitoxin titrations after combined vaccination were similar to those obtained when pulpy kidney toxoid alone was used (Hepple *et al.*, 1959).

Trials in Horses

The first trial, on 6 horses, was carried out for us by the Equine Research Station in Newmarket. The object, besides antigenic evaluation of the toxoid, was primarily to study the local reactions that largely contribute towards the prejudice against the use of aluminium hydroxide adsorbates for horses. Six horses (Thoroughbreds and ponies) were divided into 3 pairs, and each pair were injected with one, 2, and 3 ml. of the same adsorbate; thus the amounts of aluminium hydroxide responsible for the local reactions were doubled in each consecutive pair.

The antigenic responses, as shown in Table III subgroup I, was satisfactory. In 2 horses, "Emption" and "Lionelle," the presence of antitoxin was demonstrated before the first injection; in the absence of information about their accurate immunological history they served in this trial chiefly for evaluation of local reactions. Since none was observed in any horse, a further clinical trial was undertaken on other horses that became available.

Trials on Direct Challenge

Realising that the most reliable criterion for full protection of immunised animals lies in the resistance to direct challenge, and to prove conclusively that protection afforded to animals by the highly purified toxoid used in these investigations was complete, we decided to include the method of direct challenge, using toxin for that purpose. In these trials guinea-pigs and sheep were used.

Guinea-pigs

Three groups of guinea-pigs, each of 10 animals, were used. The animals of Group 1 were immunised with aluminium hydroxide adsorbed toxoid, as used in these investigations, and those of Group 2 received, for comparison, toxoid of the same concentration as used for Group 1, but unadsorbed; Group 3 served as unvaccinated controls. The animals of both immunised groups received 2 injections of toxoid at 3 weeks' interval. Two weeks after the second injection the animals were bled for antitoxin titration, and 2 days later were challenged with 3 MLD of toxin for the control animals and with 6 MLD of toxin for all the immunised animals.

All guinea-pigs vaccinated with purified adsorbed toxoid survived without showing any symptoms, 50 per cent. of the animals immunised with unadsorbed toxoid developed tetanus, and all the controls died of tetanus within 5 days of toxin injection.

TABLE III
TETANUS ANTITOXIN TITRATIONS OF HORSES IMMUNISED WITH ALUMINIUM HYDROXIDE ADSORBED TOXOID

Sub-group	Horse	Pre-immunisation	After first and before second injection	Three weeks after the second injection	Interval between injections	
I	Emption	0·1	1·0–2·0	4·0– 8·0	4 weeks	
	Lionelle	0·3	4·0–8·0	4·0– 8·0	"	
	Troytrap	<0·01	0·2–0·4	1·6– 4·0	"	
	Penny	<0·01	5·0–8·0	16·0	"	
	Princess	<0·01	0·1–0·2	1·6	"	
	Gert	<0·01	0·5–1·0	1·6– 4·0	"	
II	R.M. mare	<0·01	0·1–0·2	2·5– 5·0	4 weeks	Immunised in late pregnancy
	Topper	<0·01	0·02	5·0–10·0	"	
	Pip	<0·01	0·02	1·0– 2·5	"	
	Bony	<0·01	0·4–1·0	10·0	"	
	Lass	<0·01	0·2–0·4	1·0– 2·5	"	
	Smokey	<0·01	1·0	1·0– 2·5	3 weeks	
	Carefree	<0·01	0·2–0·5	2·5	"	
	Sally	<0·01	0·5–1·0	2·5	"	Small swelling at site of injection
	Zan	<0·01	0·1–0·2	2·5	"	
	Biz	<0·01	0·5–1·0	1·0	"	

Ten horses, including one pregnant mare and 2 Thoroughbreds, were each given intramuscularly, on the side of the neck, 2 doses of 2 ml. aluminium adsorbate. Only one of the Thoroughbreds showed a small swelling at the site of injection. This was not painful and disappeared in 2 days' time. None of the remaining horses showed any reaction, local or systemic. The results for antitoxin titrations of these horses, included in Table III subgroup II, were satisfactory.

Sheep

Eleven ewes immunised during these investigations with tetanus toxoid only, and subsequently used for study of the transfer of maternal immunity to the offspring, were challenged with 6 to 12 MLD of tetanus toxin on completion of the experiment 7 to 8 months after immunisation. At the same time 5 unimmunised controls of similar age were exposed to 3 to 5 MLD of toxin. The toxin was injected intramuscularly into the hind leg.

TABLE IV
DIRECT CHALLENGE OF SHEEP

Number of animal	Antitoxin titre at the time of challenge—A.U.	Challenge after immunisation	Challenging dose—MLD	Results
		Immunised Animals		
A9	0·1 –0·2	8 months	12	Transitory paralysis of the injected leg
A5	0·02–0·04	7 „	10	„ „ „ „
A6	0·1 –0·2	7 „	8	No symptoms
A8	0·1 –0·2	7 „	8	„
A10	0·1 –0·2	8 „	8	„
A11	0·25–0·5	7 „	7	„
A12	0·4	7 „	7	„
A13	1·0 –2·0	8 „	7	„
A17	0·4 –1·0	8 „	6	„
A18	0·4	7 „	6	„
A19	0·05–0·1	7 „	6	„
		Controls Not Immunised		
C1	<0·02	—	3	Died of tetanus within 4 days
C2	<0·02	—	3	„ „ „ „
C6	<0·02	—	3	„ „ „ „
C8	<0·02	—	4	„ „ „ „
186	<0·02	—	4	„ „ „ „

Titrations of antitoxin in the immunised ewes during the immunity trials are recorded in Table II, antitoxin titrations in immunised and control animals at the time of challenge in Table IV.

Of the 11 immunised animals, only ewes A9 and A5 receiving the highest challenging dose of 12 and 10 MLD of toxin, respectively, developed paralysis of the injected leg only, remaining otherwise normal, and these symptoms gradually disappeared. The remaining 9 immunised ewes survived the massive challenge without showing any symptoms.

All the 5 control sheep developed tetanus within 4 days' time and died or were killed *in extremis*.

Discussion

The appearance of antitoxin in the blood after tetanus toxoid injection is a proven serological manifestation of immunity response.

It has, however, been amply demonstrated by various workers that the gradual decline and eventually total disappearance of antitoxin from circulating blood does not necessarily indicate a non-immune state. Actively immunised animals without detectable antitoxin in their blood will, on challenge, respond with rapid and massive release of antitoxin, thus indicating a state of existing immunity.

This power to counteract infection is independent of the amount of free antitoxin in the blood at the time of exposure and can extend over years. Nevertheless, it has been shown that a prolonged period after initial immunisation might delay the re-appearance of antitoxin after new stimuli and might reduce the required speed (Chertkova & Shain, 1957). From the clinical point of view it is important to maintain immunological alertness at the highest level. This is achieved first, by giving a sufficiently strong initial stimulus by means of 2 injections of potent toxoid; secondly, by periodic single booster injections, called more aptly by French workers *injection rappel* (recall injection). Such injections invariably bring about a sharp rise in antitoxin titre, as a rule much higher than the titre attained after the primary immunisation and also independent of the titre initially attained. Though these periodic recall stimuli are essential, there is no more any need to adhere slavishly to fixed programmes provided the intervals between injection are not too long. Several workers emphasise the particular importance of the method of preparing toxoids in its bearing on their antigenic value.

With concentrated tetanus toxoid, purified in the same manner as for human tetanus prophylactics and adsorbed on aluminium hydroxide, constant and highly satisfactory responses were obtained in all the animals used. Not a single animal in these trials failed to respond to the injection of the adsorbate used. When the purified tetanus toxoid was combined with purified pulpy kidney toxoid for combined active immunisation of sheep, it was found that both these antigens were fully compatible, retaining the complete individuality of each, and that antigenic responses to combined preparation were similar to those obtained with each component separately.

For immunising horses, it was found that by adsorbing concentrated tetanus toxoid on a much smaller quantity of aluminium hydroxide it was possible on the one hand to retain the antigenic superiority of aluminium hydroxide adsorbate and on the other to eliminate undesirable local reactions in this species.

Exposure of the immunised animals to the direct challenge of toxin 7 to 8 months after immunisation demonstrated conclusively that the protection attained was both durable and complete, thus proving that purification and concentration of the toxoid had not reduced its immunogenic qualities. All the actively immunised animals showed a remarkable degree of resistance to the massive challenging doses of toxin, whereas all the control animals died of tetanus.

Acknowledgments.—We wish to thank Mr. Wm. C. Miller and the staff of the Equine Research Station, Newmarket, for conducting the initial trial

(*Continued at foot of col. 1 overleaf*)

Studies on Strains of Haemolytic *Escherichia coli* Isolated from Normal Swine after Weaning*

BY

S. G. CAMPBELL

Division of Bacteriology, Ontario Veterinary College, Guelph, Canada

SUMMARY.—1. *The distribution of haemolytic* Escherichia coli *organisms in rectal swabs from normal pigs is described. From* 293 *pigs,* 127 *strains of haemolytic* E. coli *were isolated.*

2. *The distribution of specific serotypes of haemolytic* E. coli *in normal pigs is discussed. The specific serotypes concerned are those commonly found in association with oedema disease in Canada. Of the* 293 *pigs sampled,* 55 *were found to possess one of these specific serotypes as part of their bacterial flora.*

3. *The implications of these findings are discussed with reference to the pathogenesis and diagnosis of oedema disease.*

Introduction

SCHOFIELD and Davis (1955) mentioned that a heavy growth of haemolytic *E. coli* had been noticed in faecal cultures made from cases of oedema disease in swine. This was the first indication that haemolytic *E. coli* might be associated with this condition and it received some support when Gregory (1955) reported that he had found haemolytic coliform organisms in 5 different outbreaks of oedema disease. Gregory also investigated the intestinal flora of normal swine and swine which died from conditions other than oedema disease and failed to find haemolytic *E. coli* organisms in the majority of pigs, though Gitter (1957) reported that these organisms could be recovered from pigs in conditions other than oedema disease. Timoney (1956) successfully protected pigs from the experimental reproduction of oedema disease by using hyper-immune serum prepared with haemolytic coliform organisms isolated from a case of oedema disease.

Sojka, Erskine, and Lloyd (1957) found that 2 specific serotypes of haemolytic *E. coli* were associated with cases of oedema disease and the in-contact litter mates of these cases. They reported that these serotypes were not common in pigs except in association with oedema disease and concluded that the disease was an entero-toxaemia, the toxin being produced by these specific serotypes of haemolytic *E. coli*. In America, Ewing, Tatum, and Davis (1958) found that the strains of haemolytic *E. coli* isolated from swine with oedema disease fell into 2 serotypes. Quinchon, Henry, and Henry (1958) in France also found 3 serotypes of haemolytic *E. coli* in cases of oedema disease. These authors considered that *E. coli* is an ordinary inhabitant of the intestinal tract and becomes pathogenic under the influence of external conditions.

In Canada, Kelen, Campbell, and Barnum (1959) reported that at least 3 serotypes of haemolytic *E. coli* were associated with oedema disease, and of these, 2 serotypes, 0138:K81 and 0139:K82, are found in Great Britain, the United States, and Canada. The third Canadian serotype and a third British serotype have an 0 antigen in common but a different K antigen structure, the former was thus tentatively designated 0141:Kx. The present author has also found serotype 0138:K81 in association with twelve outbreaks of acute gastro-enteritis and septicaemia in recently weaned pigs in the 8- to 14-week age group.

This study was carried out in an attempt to determine the distribution of haemolytic *E. coli* organisms in normal pigs in the "oedema disease age group," since it was suspected that these organisms had a widespread distribution in normal pigs and a knowledge of their incidence in normal pigs would enable one to assess their importance when found in pigs associated with any disease condition. The present study was also conducted to determine if the specific

* Abstracted from a thesis accepted by the School of Graduate Studies, University of Toronto, in partial fulfilment of the requirements for the degree of Master of Veterinary Science.

Active Immunisation of Sheep, etc.—*Concluded.*

on horses, Mr. I. E. Addison for tetanus antitoxin titrations, and Mr. R. B. French for ε-antitoxin titrations.

References

BECKER, M., & PILZ, H. (1955). *Arch. Exp. Vet. Med.* **9**. 648-58.
CHERTKOVA, F. A., & SHAIN, E. S. (1957). *J. Microbiol. Epidemiol. Immunobiol.* **28**. 209-13.
HEINIG, A. (1954). *Arch. Exp. Vet. Med.* **8**. 394-403.
HEPPLE, J. R., CHODNIK, K. S., & PRICE, E. K. (1959). *Vet. Rec.* **71**. 201-6.
LEVINE, L., STONE, J. L., & WYMAN, L. (1955). *J. Immunol.* **75**. 301-7.
MCCOMB, J. A., & TRAFTON, M. Z. (1950). *New England J. Med.* **243**. 442-4.
MORI, F., & RICHTER, S. (1951). *Dtsch. Gesundheitswes.* **6**. 1,093-109.
RAMON, G. (1924). *C. R. Acad. Sci.* **178**. 1,436-8.
———, & LEMETAYER, E. (1931). *C. R. Soc. Biol.* **107**. 1,261-2.
———. (1949). *Schweiz. Med. Wschr.* **79**. 918-9.
SAXINGER, G., & HEINIG, A. (1951). *Mh. Vet. Med.* **6**. 434-7.
SNEATH, P. A. T., KERSLAKE, E. G., & SCRUBY, F. (1937). *Amer. J. Hyg.* **25**. 464-76.
WOLTERS, K. L., & DEHMEL, H. (1940). *Z. Hyg. Infektkr.* **122**. 603-12.
———, & ———. (1942-3). *Ibid.* **124**. 326-32.
———, & FISCHOEDER, E. (1953). *Arztl. Wschr.* **8**. 537-40.

serotypes 0138:K81, 0139:K82, and 0141:Kx could be isolated from normal pigs. If they did not occur in normal pigs then the finding of pure or nearly pure cultures of these three specific serotypes of haemolytic *E. coli* in cases of oedema disease would be useful in the diagnosis of this condition.

Materials and Methods

Oedema disease occurs classically in recently weaned pigs, therefore normal pigs were selected in the 8- to 16-week age group, pigs being weaned at approximately 8 weeks of age in Ontario. Normal pigs were obtained from 3 sources. Herd A was a Government-owned piggery. Here 82 pigs in 11 pens were sampled. The housing and husbandry of these animals were very good and examination of the herd history revealed that the last case of oedema disease had occurred 10 months previously. Herd B, was also a Government-owned piggery where pigs were kept under conditions of management similar to those in herd A. Here 68 pigs distributed in 7 pens were sampled and the herd history revealed that no cases of oedema disease had occurred in the previous 6 months. Both herd A and herd B had been under careful veterinary supervision for a number of years and therefore accurate herd histories were obtainable. The third survey was conducted at a public livestock market, market C. It was thought that pigs passing through this market would provide a good random sample of the pig population of Southern Ontario, although the incidence of oedema disease in herds from which these pigs had come was not known and their ages were merely estimated to be between 8 and 16 weeks. All the pigs which were sampled appeared to be healthy. 143 pigs distributed in 23 pens and thus representing 23 farms were sampled.

A rectal swab was taken from every pig, plated on sheep blood agar, and incubated aerobically at 37° C. overnight. Next day the plates were examined for colonies of haemolytic *E. coli* and estimations were made of the number of haemolytic *E. coli* colonies present relative to the colonies of all other types of bacteria on the plate. On this basis the numbers of haemolytic *E. coli* colonies were divided into three groups, *i.e.* 1 to 40 per cent., 40 to 80 per cent., and 80 to 100 per cent. Inclusion in the 1 to 40 per cent. group meant that on primary isolation colonies of haemolytic *E. coli* were in the minority, while inclusion in the 80 to 100 per cent. group meant that haemolytic *E. coli* were in pure or practically pure culture. Representative colonies were selected and sub-cultured on to tryptose agar slopes for storage until biochemical and serological tests could be carried out.

All the strains included in this report conformed to the classical definition of *E. coli* as outlined by Kauffmann (1951) and in addition had a wide clear zone of haemolysis round each colony. All strains of haemolytic *E. coli* isolated were submitted to serological examination using three OK diagnostic antisera. These antisera had been prepared in rabbits against the three serotypes of haemolytic *E. coli*, 0138:K81, 0139:K82, and 0141:Kx which have been found to be associated with oedema disease in Canada. The materials and methods for the production, standardisation, and use of these antisera were the same as those recommended by Edwards and Ewing (1955). The indentification of unknown strains of haemolytic *E. coli* was made on the basis of their "O" and "K" antigens, using rapid slide agglutination tests for preliminary screening and serial tube "O" and "K" agglutination tests for the final identification of strains.

Incidence of Strains of Haemolytic *E. coli* In Normal Pigs

Results

The results of the distribution of haemolytic *E. coli* from rectal swabs of normal pigs are summarised in Table I. From this table it will be seen that out of a total of 293 pigs, strains of haemolytic *E. coli* were isolated from 127 pigs representing 43 per cent. of the total number of pigs. This percentage was fairly constant within the 3 sources; since in herd A, 43 per cent. of the total had a haemolytic *E. coli* present in their faeces; in herd B, 44 per cent. had a haemolytic *E. coli* present in their faeces; at market C, 43 per cent. had a haemolytic *E. coli* present in their faeces. It is of interest to note that in herd A and herd B at least one pig in every pen had a haemolytic *E. coli* present in its intestinal flora, and at market C, 18 of the 23 pens contained pigs from which haemolytic *E. coli* organisms could be isolated.

TABLE I
DISTRIBUTION OF HAEMOLYTIC *E. coli* IN NORMAL PIGS FROM THREE SOURCES

Source	Total no. of pens	No. pigs sampled	No. with haemolytic *E. coli* present	Distribution of haemolytic *E. coli*		
				1-40%	40-80%	80-100 %
Herd A	11	82	35	16	12	7
Herd B	7	68	30	12	8	10
Market C	23	143	62	24	33	5
Total	41	293	127	52	53	22
Per cent.			43%	18%	18%	7%

Discussion

It should be noted that the above-mentioned strains of haemolytic *E. coli* were untyped and that the figures represent the numbers of haemolytic *E. coli* isolated from normal pigs without reference to specific serotypes. From the above results it appears that haemolytic *E. coli* can in fact be isolated from normal pigs and in a number of cases (7%) they are found in pure or nearly pure cultures. Campbell (1959) reported that 83 per cent. of pigs suffering from oedema disease had a haemolytic *E. coli* organism isolated from their rectal swabs and of these 43 per cent. were in pure or nearly pure culture. The incidence of a large number of haemolytic *E. coli* organisms in a rectal swab is therefore not necessarily an indication that the animal is suffering from oedema disease or any other condition, but this finding must be considered in the correct perspective with other factors such as clinical and *post-mortem* findings.

Distribution of "Oedema Disease" serotypes of Haemolytic *E. coli* in Normal Pigs

Results

The 127 strains of haemolytic *E. coli* isolated from normal pigs were typed serologically to determine if any of them belonged to the 3 serotypes commonly associated with oedema disease in Canada and elsewhere. The results of the findings are summarised in Table II. In herd A, only 4 of the 35 strains of haemolytic *E. coli* isolated could be typed using the 3 antisera and these 4 all belonged to serotype 0138:K81. In herd B, 26 of the 30 strains isolated from pigs could be identified and they also belonged to serotype 0138:K81. At market C, 62 strains of haemolytic *E. coli* were isolated from normal pigs and of these 25 could be typed, 16 belonging to serotype 0138:K81 and 9 to serotype 0141:Kx. Three strains had an "O" antigen identical to that of serotype 0139:K82 but their "K" antigen was different.

In summary, from a total of 293 pigs, 127 strains of haemolytic *E. coli* were isolated and of these 46 belonged to serotype 0138:K81, 9 to serotype 0141:Kx and the remaining 72 could not be typed using the 3 sera available. Serotype 0139:K82 was not isolated from any normal pigs.

TABLE II
DISTRIBUTION OF "OEDEMA DISEASE" SEROTYPES AMONG NORMAL PIGS

Source	No. pigs sampled	No. pigs with haem. *E. coli* present	Distribution of specific serotypes			
			0138 : K81	0139 : K82	0141 : Kx	un-known
Herd A	82	35	4	0	0	31
Herd B	68	30	26	0	0	4
Market C	143	62	16	0	9	37
Total	293	127	46	0	9	72

Table III was constructed to demonstrate the distribution of specific serotypes in pens which contained normal pigs. In herd A, 27 per cent. of the pens contained at least one pig harbouring serotype 0138:K81 in its intestinal flora. In herd B, 100 per cent. of the pens had serotype 0138:K81 present in at least one pig and at market C, 30 per cent. of the pens contained the serotypes 0138:K81 or 0141:Kx.

TABLE III
DISTRIBUTION OF "OEDEMA DISEASE" SEROTYPES IN PENS CONTAINING NORMAL PIGS

Source	No. pens sampled	No. pens with haem. *E. coli* present	No. pens with 0138 : K81 or 0141 : Kx	Per cent. pens with 0138 : K81 or 0141 : Kx
Herd A	11	11	3	27%
Herd B	7	7	7	100%
Market C	23	18	7	30%

A study of Table II will reveal that two of the "oedema disease" serotypes do in fact occur in normal pigs and from Table III it will be seen that in herd B one isolation of serotype 0138:K81 could be made in every pen sampled. The incidence of these serotypes in pens was lower in herd A (27%) and at market C (30%). A study of the relative numbers of haemolytic *E. coli* found in the original isolates revealed those of the 55 strains of serotypes 0138:K81 and 0141:Kx isolated from normal pigs; 19 strains occurred in the 1 to 40 per cent. group, 21 strains in the 40 to 80 per cent. group and 15 in the 80 to 100 per cent. group. It is thus apparent that these 2 serotypes, which occur frequently in pigs with oedema disease, can sometimes be isolated in practically pure culture from normal pigs. Since there was no history of the recent occurrence of oedema disease in herds A or B these pigs could certainly not be classed as pigs in contact with oedema disease.

Discussion

In pigs which have died of oedema disease these specific serotypes of haemolytic *E. coli* are usually found in pure or nearly pure culture from the intestinal tract. Campbell (1959) found that in pigs with oedema disease haemolytic *E. coli* were found most consistently in the colons and rectums and he concluded that the bacteriological findings in these viscera were comparable. Rectal swabs were thus used as the basis for comparison between normal pigs and pigs with oedema disease in the present study. It appears therefore, that, whereas specific serotypes of haemolytic *E. coli* are found in association with oedema disease in Canada, two of these serotypes 0138:K81 and 0141:Kx are sufficiently well distributed in normal pigs and on occasions in practically pure culture, that one might suspect that the pathogenesis of the condition of oedema disease is dependent upon an additional factor which permits the proliferation of these specific serotypes. Their presence in normal pigs may indicate that predisposing factors such as change in feeding, change in housing or a non-specific change in environment are still essential ingredients in the pathogenesis of this condition. Normal pigs harbouring the specific serotypes of haemotic *E. coli* formerly associated only with oedema disease may possess a different degree of immunity to these specific serotypes, than do pigs which develop oedema disease. These factors should not be overlooked in the further study of oedema disease.

References

CAMPBELL, S. G. (1959). M.V.Sc. Thesis. University of Toronto.

EDWARDS, P. R., & EWING, W. H. (1955). "Identification of Enterobacteriaceae." Burgess Publishing Company, Minneapolis, Minnesota.

EWING, W. H., TATUM, H. W., & DAVIS, B. R. (1958). *Cornell Vet.* **48**. 201.

GITTER, M. (1957). *Brit. Vet. J.* **113**. 168.

GREGORY, D. W. (1955). *Vet. Med.* **50**. 609.

KAUFFMANN, F. (1951). "Enterobacteriaceae." 1st ed. Munksgaard, Copenhagen.

KELEN, A. E., CAMPBELL, S. G., & BARNUM, D. A. (1959). *Can. J. Comp. Med.* **23**. 216.

QUINCHON, C., HENRY, M., & HENRY, G. (1958). *Rec. Med. Vet.* **134** 437.

SCHOFIELD, F. W., & DAVIS, D. (1955). *Can. J. Comp. Med.* **19**. 242.

SOJKA, W. J., ERSKINE, R. G., & LLOYD, M. K. (1957). *Vet. Rec.* **69**. 293.

TIMONEY, J. F. (1956). *Ibid.* **68**. 849.

The Complement Fixation Test as a Measure of Resistance to Dictyocaulus Infection

BY

J. F. MICHEL

Ministry of Agriculture, Central Veterinary Laboratory, Weybridge

AND

R. L. CORNWELL

Department of Veterinary Preventive Medicine, University of Liverpool

SUMMARY.—Ten pairs of calves were used to compare the level of acquired resistance to reinfection with D. viviparus *and levels of complement fixing antibody in the blood as determined by means of a heated whole-worm antigen. Antibody titres required considerably longer to develop than did resistance to reinfection and no correlation between the two measurements could be demonstrated.*

IT was shown by Stewart (1950) that complement fixing antibodies can be demonstrated in the blood of animals infected with trichostrongylid nematodes. More recently a complement fixation test using a heated antigen made from adult worms by a procedure similar to that of Stewart has been used by Jarrett, Jennings, McIntyre, Mulligan and Urquhart (1955) to demonstrate antibodies in the blood of calves infected with *Dictyocaulus viviparus*. These workers concluded that "In parasitic bronchitis there are humoral antibodies which are detectable and measurable by a complement fixation test and the serum containing these antibodies confers a considerable degree of protection." Certainly, if it could be shown that these complement fixing antibodies were themselves protective or if their level was at all times indicative of the resistance of the calf, a serological test of this nature would be of the greatest value. It would not be justifiable, however, to assume that this is necessarily the case. The manifestations of resistance behave as though they are to some extent independent of each other, and it may well be that the appearance of complement fixing antibodies in the blood is, in a similar manner, independent of the manifestations of resistance.

To determine whether complement fixing antibodies are protective or at least whether their level can be taken as a measure of protection, resistance to reinfection must be determined helminthologically and compared with the level of complement fixing antibody in blood taken at the time of challenge. This note presents the results of such a comparison.

Methods

Resistance to the establishment of worms was determined by means of a comparison between the number of worms established in a resistant calf and the number established in a susceptible control calf when both were given the same number of infective larvae. From these worm counts a quantitative measure of protection can be calculated. The formula employed,

$$P = 100\left(1 - \frac{\text{worms from resistant calf}}{\text{worms from control calf}}\right)$$

is useful in that it provides a scale of values from 0 for a fully susceptible animal to 100 for an entirely refractory one.

Jersey calves bought when a few days old were reared in conditions designed to prevent accidental infection with lungworms. They were arranged in pairs and when about 3 months old, one calf of each pair was infected with *D. viviparus*, a dose of either 3,500 or 3,200 3rd-stage larvae being administered orally. After different intervals of time a second and larger dose of 35,000 or 40,000 larvae was given to both animals of the various pairs. The calves were killed 10 days after receiving this large dose and the worms were recovered from their lungs. In the case of the previously infected calves the worms attributable to the second infection, which could be distinguished on the basis of size, were counted separately.

Both calves of each pair were bled weekly from the time that one of them received the first dose of larvae.

Complement fixation tests were carried out on the 50 per cent. end point basis using a serum dilution method. A standardised 3 per cent. suspension of sheep red blood cells and 3 units of complement were employed. The antigen was prepared from adult worms by the method of Stewart (1950).

The titre of any serum is expressed as the reciprocal of the dilution at which the end point occurred. Adequate controls including standard known negative and positive sera were set up with each batch of tests.

Results

The numbers of worms recovered from the 10 pairs of calves used are shown in Table I together with values for "P" which have been calculated for each. The antibody titre of the last blood sample taken before the challenge dose was given is also shown and is plotted against "protection" in Fig. 1. It will be seen that there is no correlation between protection and antibody titre.

The course of antibody titre is illustrated in Fig. 2 which shows the record of all those infected animals which were killed more than 80 days after the first infection. Consistently negative results were obtained with the sera of the control calves apart from an occasional titre of 5 and one of 10 which were regarded as non-specific.

TABLE I

Pair No.	Days from 1st-2nd infection	Worms of challenge dose from resistant calf	Worms from control calf	P	Titre
1	11	2,760	9,602	71·3	10
2	12	2,539	9,697	73·8	0
3	15	6,022	8.689	30·7	0
4	15	3,036	9,372	67·6	0
5	18	884	7,361	88	5
6	85	1,627	9,160	82·3	80
7	91	7,062	9,514	25·8	80
8	105	2,771	9,570	71	40
9	105	691	9,748	92·9	160
10	135	6,364	6,916	8	80

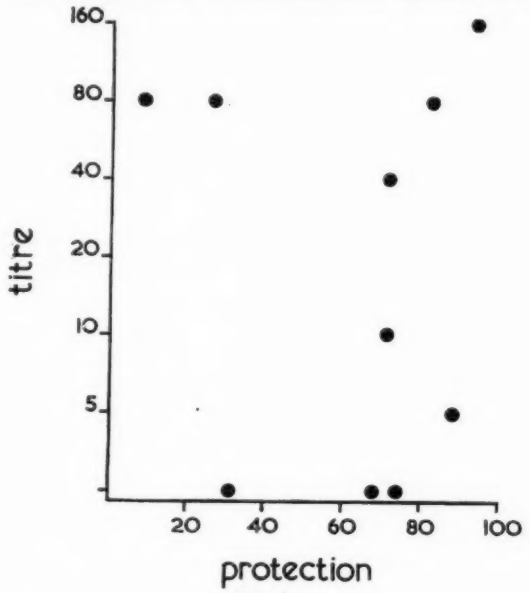

FIG. 1.

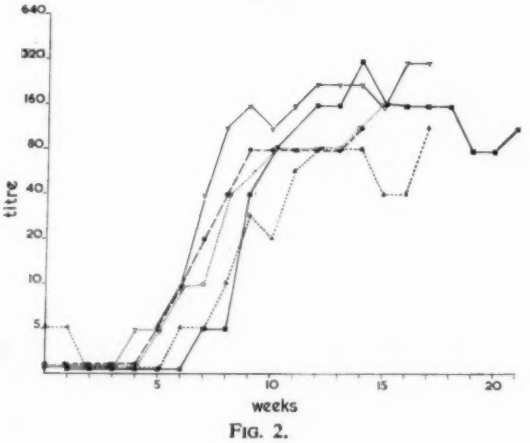

FIG. 2.

Discussion

It will be seen from Table I that cases of low titre associated with strong protection occurred in animals challenged a short time after the first infection and it appears both from this and other experiments that a useful degree of protection tends to develop some weeks before a rise in antibody level is apparent.

At a later stage in the infection a high titre was associated with strong protection in 3 out of 5 pairs challenged 85 to 135 days after the initial infection, but a similar titre was associated with low protection in the remaining 2 pairs. It is concluded that where a high titre and strong protection occur in the same animal there is no direct connexion.

The lack of correlation between titre and protection shown by this experiment to occur at different stages in the infection must suggest that in the case of *D. viviparus* infection complement fixing antibodies as measured by a test using a heated adult worm antigen are not themselves protective nor can their level be used as a reliable measure of an animal's ability to prevent the establishment of worms.

Antibody levels measured by the procedure described here may prove to be related to some other manifestation of resistance. Similarly it is possible that antibodies detected with the aid of antigens prepared by other methods or from other stages of the parasite might show a better correlation with protection.

Acknowledgments.—We are indebted to Professor E. G. White for his interest in this work. Thanks are also due to Miss J. Berry for technical assistance.

References

JARRETT, W. F. H., JENNINGS, F. W., MCINTYRE, W. I. M., MULLIGAN, W., & URQUHART, G. M. (1955). *Vet. Rec.* **67**. 291.

STEWART, D. F. (1950). *Austr. J. agric. Res.* **1**. 285.

SWINE FEVER: WITHDRAWAL OF RESTRICTIONS IN KENT

The Ministry of Agriculture has just announced that as from December 13th the Infected Area restrictions affecting the movement and marketing of pigs in the central and eastern parts of Kent are being withdrawn.

During the period October 12th to December 8th, 1959, the following outbreaks have been confirmed in Kent:—

Period	*No. of Outbreaks*
October 12th to October 15th	4
October 16th to October 31st	8
November 1st to November 15th	3
November 16th to November 30th	2
December 1st to December 8th	1

News and Comment

THE VETERINARY RECORD

THE VETERINARY RECORD for December 26th will be closing for press two days earlier than usual to meet Christmas printing and postal arrangements. Contributors and advertisers who wish to publish material in this issue are asked to see that it reaches the editorial offices *not later than Friday, December 18th.*

NEW SERIES OF RADIO PROGRAMMES ON "THE CAT"

Following the successful radio series on "The Dog" comes another on "The Cat," also in Network Three. It will deal with the cat from all aspects, including the wild and domestic types and the training, showing and care of the latter—the "harmless necessary cat," as Shakespeare described it. This series is again being arranged in collaboration with the Animal Health Trust, and Dr. W. R. Wooldridge will take part in several of the programmes. They will be broadcast on Mondays from December 28th; the first two at 7.30 p.m. and the rest at 6.50 p.m.

In the introductory programme Dr. Wooldridge and Dr. E. C. Appleby, of the Department of Pathology, Royal Veterinary College, London, will discuss the history and evolution of the cat, its nature and habits, and the development of modern breeds. Dr. R. A. Green, Lecturer in Veterinary Anatomy at Cambridge, will follow with a talk on "The Body of the Cat," with a reference to psychology. "The Voice and Manner of the Cat," by Dr. F. R. Bell, of the Royal Veterinary College, will be illustrated with recordings of all kinds of cat noises.

Alex Kerr, chief lion trainer in Bertram Mills's Circus, will take part in the fourth programme with Dr. Appleby, in which the question will be discussed: "Why can 'big cats' be trained to perform in circuses, but domestic cats rarely learn parlour tricks?" In the fifth programme Miss Joan Joshua will talk about feeding and caring for the domestic cat, and finally Dr. Wooldridge, with a leading member of the cat fancy, will discuss shows and give advice on breeding for show purposes.

FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease has been confirmed among cattle and pigs at Mitcheldean, Gloucs.; Roath Cattle Market Slaughterhouse, Cardiff; Leicester City Abattoir and Great Bowden Market Harborough, Leics. (within the existing Infected Area around Hallaton); and among pigs at Westhaughton, nr. Bolton, Lancs; also among pigs at Saul, Gloucs.; among cattle at Hallaton, Market Harborough, Leics.; and among cattle at Barnston, nr. Nottingham; also among pigs at Heaton Norris, Stockport, Cheshire.

Special orders have been made declaring the administrative county of Gloucestershire, including the whole of the city and county borough of Bristol, and the administrative county of Leicestershire, including the city and county borough of Leicester, to be Controlled Areas to prevent the spread of foot-and-mouth disease.

This step has been taken because animals exposed to infection were sold in Gloucester market last week and in Leicester market on November 26th and December 3rd. Special action is necessary to stop the further movement of animals and to facilitate the tracing and examination of them.

The restrictions came into force at midnight on December 7th, and will remain in force until the risk of spread of infection is past.

The restrictions in the Controlled Area will include:—

(i) The prohibition of movement of animals out of the Area;

(ii) Animals may be moved into the Area under licence (a) to a slaughterhouse; (b) to a farm where animals must be detained for 14 days;

(iii) Within the Area all movements will be under licence but animals may be moved direct by railway through a Controlled Area without a licence provided that they are not untrucked within it;

(iv) The holding of store markets will be prohibited;

(v) Animals sent to markets must be sent on direct to slaughterhouses or if unsold returned direct to the farm they came from and detained for 6 days.

Movement licences will be issued by the inspector appointed by the local authority who is usually a police officer.

SWINE FEVER : WITHDRAWAL OF RESTRICTIONS IN LANCASHIRE AND CHESHIRE

As from December 7th, the infected area restrictions affecting the movement and marketing of pigs in the central and southern parts of Lancashire and the whole of Cheshire have been withdrawn. The swine fever situation in these counties no longer warrants the maintenance of these restrictions which were imposed in order to reduce the risk of spread of the disease.

During the period September 6th to December 2nd, 1959, the following outbreaks have been confirmed in Lancashire and Cheshire:—

Period	*No. of Outbreaks*	
	Lancashire	*Cheshire*
September 6th to September 15th	4	6
September 16th to September 30th	8	7
October 1st to October 15th	5	4
October 16th to October 31st	2	1
November 1st to November 15th	8	2
November 16th to November 30th	5	2
December 1st to December 2nd	—	1

WARNING TO STOCKOWNERS AND POULTRY-KEEPERS

Arising out of investigations into the origin of recent outbreaks of foot-and-mouth disease, fowl pest and swine fever, there is circumstantial evidence that primary cases have arisen through animals eating uncooked waste food.

The Ministry of Agriculture has issued a warning

to stockowners and poultry-keepers that scrupulous attention must be paid to the rule that no person may feed unboiled waste food to animals or poultry, or allow them to have access to it.

THE R.C.V.S. LIBRARY

In order to have as few books as possible in the post during the Christmas rush no parcels will be despatched from the library after Friday, December 18th, until after the holiday.

The library will close at 1 p.m. on December 24th and re-open on Tuesday, December 29th, at 10 a.m.

PERSONAL

Mr. Tom Stewart, M.R.C.V.S., D.V.S.M., has been appointed to the new post of General Manager of the Veterinary and Farm Sales Division of Bayer Products Ltd., Kingston-upon-Thames.

Mr. I. W. Lesslie, of the Central Veterinary Laboratory, Weybridge, is attending the International Colloquium on Mycobacteria to be held at the Prince Leopold Institute of Tropical Medicine in Antwerp from December 4th to 6th, where he is contributing a paper entitled "Purified Protein derivatives prepared from non-tuberculous mycobacteria including *Mycobacterium johnei*." Mr. Lesslie is also paying a short visit to the Central Veterinary Institute, Rotterdam.

Births

CARTER.—On November 23rd, 1959, at 2 Bedford Villas, Tavistock, Devonshire, to Christine Carter, B.D.S., L.D.S., R.C.S., wife of George Carter, B.V.SC., M.R.C.V.S., a daughter, Rachel, sister for Peter and Timothy.

FELL.—On December 6th, 1959, at 33 Belvidere Street, Aberdeen, to Margaret, wife of Bernard Fell, M.R.C.V.S., a son, Nicholas Hugh, brother for Susan and Jessica.

NEIL.—On November 19th, 1959, to Lillian, wife of H. J. D. Neil, M.R.C.V.S., at Lansdown, 3 Lidden Road, Penzance, a daughter, Amanda Holly.

SACHS.—On September 27th, 1959, to Sheila, wife of Max Sachs, M.R.C.V.S., of Kfar Vitkin, Israel, a son, David, brother for Harold and Jonathan.

COMING EVENTS

December

6th (Sun.). Meeting of the Kenya Veterinary Association at Uplands Bacon Factory and Sports Club, 11 a.m.

15th (Tues.). Special Meeting of Council of the Royal College of Veterinary Surgeons at 10 Red Lion Square, London, W.C.1.

16th (Wed.). Meeting of the Sussex Veterinary Society at the Old Ship Hotel, Brighton, 2.30 p.m.

17th (Thurs.). Meeting of the South Eastern Veterinary Association at the Royal Star Hotel, Maidstone, 2.30 p.m.

Joint Meeting of the Society of Practising Veterinary Surgeons and the Western Counties Veterinary Association at the Rougemont Hotel, Exeter, 2.30 p.m.

18th (Fri.). Winter Meeting of the North of England Veterinary Medical Association in the Agricultural Lecture Room, King's College, Newcastle-upon-Tyne, 1, 2.30 p.m.

January, 1960

6th, 7th and 8th (Wed., Thurs. and Fri.). Committee and Council Meetings of the Royal College of Veterinary Surgeons at 10 Red Lion Square, London, W.C.1.

13th (Wed.) Meeting of the Southern Counties Veterinary Society at the Royal Hotel, Winchester.

BRITISH VETERINARY ASSOCIATION

Quarterly Meetings in London

Tuesday, January 19th, 1960, at 7 Mansfield Street.
5 p.m. Finance Sub-committee.

Wednesday, January 20th, at 7 Mansfield Street.
10 a.m. Farm Livestock Committee.
11.30 a.m. Parliamentary and Public Relations Committee.
2.15 p.m. Veterinary State Medicine Committee.
4.30 p.m. Home Appointments Committee.

Thursday, January 21st, at 7 Mansfield Street.
10 a.m. Small Animals Committee.
2.15 p.m. General Purposes and Finance Committee.

Friday, January 22nd, at Connaught Rooms, W.C.2.
10.15 a.m. Council Meeting.

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax

Ayr. Shacklehill Farm, Mossblown, Tarbolton, Mauchline (Dec. 4).

Renfrew. East Fulton Farm, Linwood, Paisley (Dec. 3).

Foot-and-Mouth Disease

Ches. Norris Hill Farm, Heaton Moor, Stockport (Dec. 7).

Glam. Roath Cattle Market, Slaughterhouse Lairages, Cardiff (Dec. 2); Roath Cattle Lairage, Slaughterhouse, Cycle Street, Cardiff (Dec. 3); Public Slaughterhouse, Court Road, Barry (Dec. 5).

Glos. Bilbut Farm, Mitcheldean (Dec. 2); Sun Nap, Harrow Hill, Drybrook; Woodfield, Woodside, Ruardean (Dec. 3); Burnthouse Farm, Saul; Hill Farm, Painswick, Stroud (Dec. 5); Bellamy's Farm, Longney; Brook Farm, Brookthorpe (Dec. 6).

Lancs. Farnworth Close Farm, Westhoughton, Bolton (Dec. 5).

Leicester. Gables Farm, Hallaton, Market Harborough (Dec. 6); Heygate Buildings, Greenford, Great Bowden, Market Harborough; Slaughterhouse, Leicester Abattoir, Aylestone Road; Hickling Lodge, Hickling, Melton Mowbray (Dec. 7).

Notts. Barnstone Lodge, Barnstone (Dec. 5).

Fowl Pest

Beds. 27 Oakley Road, Clapham (Dec. 3).

Bucks. Abbotsbury, Hale Lane, Wendover, Aylesbury; Broughton Farm, Aston Clinton Road, Aylesbury; Beries Farm, Weston Turville, Aylesbury (Dec. 1); Spencers Meadow, Aylesbury Road, Aston Clinton, Aylesbury (Dec. 3).

Cambs. Hillcroft Creek, March; 3 The Borough, Aldreth, Ely; Odsey Way, Steeple Morden, Royston (Dec. 1); Walcot, Bridge Lane, Wimblington, March; 9 High Street, Conington (Dec. 2); 122 Badgeney Road, March; Parson Charity,

Wilburton Road, Stretham, Ely; Gaul Farm, Gaul Road, March (Dec. 3); Clovelly, Whittles Road, March; Red Bungalow, Mill Road, Over; Gravel Farm, Twenty pence Road, Cottenham; 81 Elm Road, March; St. Louis Culver, Cambridge Road, Coton (Dec. 4); 19 Newmarket Road, Stretham, Ely; 3 Council Houses, The Footpath, Harston; School Farm, Chittering; 17 Bridge Lane, Wimblington, March; The Green, Newton; Duke of Wellington, Bourn (Dec. 5); 66 Aldreth Road, Haddenham, Ely; Chear Farm, Stretham, Ely; 132 High Street, Cottenham; Flat Read Farm, Haddenham, Ely; Bleak House, Pious Drove, Upwell, Wisbech; Long Drove, Smallholding, Cottenham; The Chase, Gaul Road, March (Dec. 6); Brench Farm, Great Wilbraham; Trevordale, Pious Drove, Upwell, Wisbech; Topcliffes Mill, Meldreth, Royston; Pious Grove Farm, Upwell, Wisbech; 14 Uffen Way, Sawston; 9 Cambridge Road, Stretham, Ely (Dec. 7).

Derbys. Elms Farm, Palterton, Chesterfield (Dec. 5); Spray's Buildings, Palterton, Chesterfield (Dec. 6).

Essex. Green View, Stebbing, Dunmow (Dec. 1).

Glos. The Home Farm, Bredons Norton, Tewkesbury (Dec. 2).

Herts. Ideal Farm, Braughing Friars, Ware (Dec. 6).

Hunts. Mill Green, Warboys (Dec. 1); Wentwood Farm, St. Ives; Garden next 12, Tenter Leas, St. Ives; The Bridge Bungalow, Wood Walton; North Road, St. Ives (Dec. 2); 32 Green Lane, St. Ives (Dec. 3); 85 Ramsey Road, St. Ives; Collingwood Farm, St. Ives (Dec. 4); 5 Park Avenue, St. Ives; Fairfield, Earith Road, Colne; Ferndale, Chatteris Road, Warboys (Dec. 5).

Kent. Potteries Farm, High Halden, Ashford; Mamden Farm, Bethersden, Ashford (Dec. 2); Green Lane Land, Bethersden, Ashford (Dec. 4); Home Farm, Rusthall, Tunbridge Wells; Longs Corner Farm, Bethersden, Ashford; Langley, Bethersden, Ashford (Dec. 5); Abeden Farm, Smarden, Ashford (Dec. 6).

Leics. Manor Farm, Little Dalby, Melton Mowbray (Dec. 6).

Lincs. Sycamore Cottage, Blacksmith Lane, Navenby (Dec. 1); Greenaleigh, Skegness Road, Burgh-le-Marsh, Skegness (Dec. 2).

Middlesex. Wayfields, Chertsey Road, Shepperton; Elm Tree Farm, New Year Green, Harefield, Uxbridge (Dec. 1); Meadow View, Chertsey Road, Shepperton (Dec. 3); Wayside Farm, Dockett, Eddy Lane, Shepperton (Dec. 4); St. Leonards Farm, New Years Green, Harefield, Uxbridge (Dec. 5); Little Piers, Clay Hill, Enfield (Dec. 7).

Norfolk. 69, Quebec Road, Norwich; Thorpes Farm, New Road, Shouldham, King's Lynn; Brookside, North Elmham, Dereham; Salhouse Hall, Salhouse, Norwich; Racecourse Premises, Bawdeswell; Grange Farm, Horstead, Norwich (Dec. 1); Jocelyn Lodge, Horningtoft; Mell Road, Whitwell, Reepham, Norwich; Sweet Briar, Broomhill Lane, Reepham, Norwich; The Common, Craymere Road, Briston, Melton Constable; 9, Norwich Road, Horsham St. Faith, Norwich (Dec. 2); Millhouse Poultry Farm, North Elmham, Dereham; The Aerodrome, Rackheath, Norwich; Kerbistone Road Premises, Reepham, Norwich; Mill Road Premises, Reepham, Norwich (Dec. 3); Northall Green, Dereham; Northall Farm, Northall Green, Dereham; The Moor, Reepham, Norwich; Waveney, Wertwell, Harleston; Green Lane, Rackheath, Norwich; Norman's Farm, Salhouse, Norwich; 1, Council Houses, Main Road, Billingford, Dereham; Mill Road, Whitwell, Reepham, Norwich; The Beeches, Rackheath, Norwich; Bakenham Hall, Salhouse, Norwich; Dinsdale, Brook Road, Wortham, Diss (Dec. 4); Church Lane, Letheringsett, Holt; Cropson Hall, Heydon, Norwich; Ivy Farm, Badley Moor, Dereham; Holmlea, Station Road, Alburgh, Harleston; No. 1 Glebeway, Horstead, Norwich (Dec. 5); Horstead Farm, Horstead, Norwich; Chapel Farm, Wortwell, Harleston; 3, Sir Edward Stacey Road, Rackheath, Norwich; Highways, Rickinghall, Diss; The Old Magpies, Wortham, Diss; Halbrook Farm, Alburgh, Harleston; Clint Green Farm, Clint Green, East Dereham; Bow Lane, Reepham, Norwich; Church Farm, Billingford, Dereham; Marsh House, Wortwell, Harleston (Dec. 6); Low Farm, Morley Lane, Wicklewood, Wymondham; Holly Farm, Mattishall Road, East Dereham; Belmont, Stalham, Norwich; Harbour Lane, Garboldisham, Diss (Dec. 7).

Northants. 45, Thorpe Lea Road, Peterborough (Dec. 2).

Notts. Manor Farm, Rufford, Ollerton, Newark (Dec. 2); Longland Lane, Laying Unit, Farnsfield (Dec. 4); Wenlow Road Laying Unit, Ollerton, Newark (Dec. 5); Line Farm, Blidworth, Mansfield (Dec. 6).

Suffolk. Brook House Farm, Henley, Ipswich; Walnut Tree Farm, Henley, Ipswich; 138, Stoke Ash, Eye (Dec. 1); 5, Little Box Meadows, Little Blakenham, Ipswich: Little Box Meadows, Little Blakenham, Ipswich; School Farm, Mendlesham, Stowmarket; High House Farm, Clydon, Ipswich; Castle Street, Eye; Lingate, Newmarket; Hall Farm, Undley, Lakenheath; Mill Farm, Gosbeck, Ipswich (Dec. 2); Hall Farm, Fornham, All Saints, Bury St. Edmunds; Mount Pleasant Farm, Stowupland, Stowmarket; Slough Farm, Great Ashfield, Bury St. Edmunds; Street Farm, Badwell Ash, Bury St. Edmunds; Cross Green, Old Newton, Stowmarket; Brewery Farm, Hemingstone, Ipswich; Norman Farm, Wyverstone, Stowmarket; Meadowcroft, Old Newton, Stowmarket (Dec. 3); Home Farm, Hardwick, Bury St. Edmunds; Rose Cottage, Wyvestone, Stowmarket; The Firs, High Street, Coddenham, Ipswich; Park Farm, Wyverston; Poplar Farm, Mendelsham, Stowmarket; The Bungalow, Whitehorse Corner, Wetherinsett, Stowmarket; Braziers Hall, Stowmarket; Mills Farm, Brockford, Stowmarket (Dec. 4); Thatchways, Wyverstone, Stowmarket; Sunnyside, Walsham le Willows, Bury St. Edmunds; Cranner Green, Walsham le Willows, Bury St. Edmunds; Berghersh House Farm, Withesham, Ipswich; Old Hall Farm, Hemingstone, Ipswich (Dec. 5); Garage & Store, Hemingstone, Ipswich New Hall, Crowfield, Ipswich; The Forge, Wetheringsett, Stowmarket; 9, Hillview, Ledgate, Stowmarket; The Meadow (North side) off Mellis Common, Mellis; The Glebe, Hemingstone, Ipswich; Redhouse Farm, Topcroft, Bungay; Little Manor Farm, Topcroft, Bungay (Dec. 6); 2, The Cottage, High Street, Gislington, Eye; Kenton, Stowmarket; The Beeches, Horton, Bury St. Edmunds; Bacton Hall Farm, Haughley, Stowmarket; Potash Farm, Wyverstone, Stowmarket; The Bungalow Stores, Mellis, Eye (Dec. 7).

Sussex. Kings Court, Burwash Common, Etchingham (Dec. 1); Setters Farm, Five Oaks, Billingshurst (Dec. 3).

Warwicks. The Laurels, Long Marston, Stratford-on-Avon; Martins Corner, Pebworth, Stratford-on-Avon (Dec. 1); The Gravel Pits, Cresshill, Welford-on-Avon (Dec. 3); Cherry View, Headland Road, Welford-on-Avon (Dec. 4); New Grounds House, Ardens Grafton, Alcester (Dec. 5).

Worcs. Masty Farm, Broadway (Dec. 2); Clacks Farm, Boreley, Ombersley (Dec. 3); Tilesford Farm, Throckmorton, Pershore (Dec. 6).

Yorks. 8, Upperthorpe Villas, Killamarsh, Sheffield (Dec. 6).

Swine Fever

Anglesey. Capel Coch, Llanercymedd (Dec. 7).

Ches. Laurel Bank Farm, Clotton, Tarporley (Dec. 1).

Dorset. Droop Farm, Hazelbury Bryan, Sturminster Newton (Dec. 4).

Essex. Joy-Vic, Larkhill Road, Canewdon, Rochford (Dec. 2); Sibley Green Farm, Thaxted, Dunmow (Dec. 7).

Hants. Moorgreen Farm, West End, Southampton (Dec. 2).

Kent. Somerfield Court, Sellindge, Ashford (Dec. 4).

Lancs. Salesis Farm, Pimhole, Bury (Dec. 3); Sandyway Farm, Sandyway, Holmeswood, Rufford, Ormskirk (Dec. 7).

Leicester. 1, Bindleys Lane, Great Glen (Dec. 3).

Lincs. Corn Close, Prospect Street, Horncastle (Dec. 3).

Mont. The Farm, Middletown, Welshpool (Dec. 4); Glanhanw, Llanfair Caereinion, Welshpool; Tanybryn, Cemmacs, Machynlleth (Dec. 7).

Norfolk. The Leas, 3, Castle Acre Road, Swaffham (Dec. 2); Church Farm, Billingford, East Dereham (Dec. 3); Bridge Farm, Antingham, North Walsham (Dec. 4); Holbrook Hall Farm, Alburgh, Harleston; Sunnyside, Castleacre Road, Swaffham; Church Farm, Howe, Norwich (Dec. 7).

Somerset. Stricklands Orchard, Trott Farm, Bradford-on-Tone, Taunton; Orchard by Stonehouse Park, Hele, Bradford-on-Tone, Taunton (Dec. 7).

Letters to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

Keith Entwistle Memorial Fund

Sir,—The Cambridge University Veterinary Society wishes to thank all who have subscribed so generously to this Fund.

The total collected is now £137 6s. 6d. For any who knew Keith Entwistle and would like to add a contribution, the Fund is still open. The money collected is being invested in £5% Defence Bonds and only the interest will be used for defraying the expenses of the annual Memorial Lecture. The Society has undertaken to make up any deficit from its own slender resources.

The first Memorial Lecture will be held in 1960 and will be given by a scientist of distinction.

Yours faithfully,

R. A. GREEN.

Senior Treasurer,

Sub-Department of Veterinary Anatomy,
Tennis Court Road,
Cambridge.

December 7th, 1959.

Hydatid Cysts in Offal

Sir,—There has recently been an alarm, fortunately unfounded, that a consignment of beef intended for animal consumption was infected with anthrax. Little attention, however, is given to the ever-present danger inherent in allowing offal, particularly liver, infected with hydatid cysts to be sold for animal consumption.

Correspondence, about the sale of meat for animal consumption, with the Ministry of Health and the Ministry of Agriculture, Fisheries and Food reveals that in this country "there are no regulations relating to the sale of offal containing hydatid cysts." The Ministry of Health states: "We are of opinion that any offal containing hydatid cysts should be condemned as unfit for human consumption" but it is not apparently generally recognised that the danger is far greater to human beings if their pet dogs are fed infected raw liver than if they themselves eat it.

Not long ago I purchased, from a pet shop, cow liver which contained a large number of viable cysts, even although this particular shop had previously maintained that any meat sold by them as pet food was perfectly safe to be fed raw. The owner, when questioned by the local Public Health Inspector, revealed the fact that cysts were normally cut out before the liver was sold; he was quite unaware of the potential danger. Cutting of the viable cysts would spread the infective scoleces on to all the meat with which it came into contact.

This appears to be a fantastic and serious situation. Hydatid is not a rare disease in Britain; it causes a number of deaths annually and probably occurs more frequently that it is diagnosed. At one time it would have been regarded as a disease more likely to occur in rural areas and in persons in close contact with sheep-dogs and other working farm dogs; dogs are prohibited from abattoirs but, with the continued sale of infected offal in pet shops in urban districts, it would seem likely that the control which this would exercise would be negligible and that the incidence of this disease is likely to increase and to become more widespread throughout the country.

Is it not time that legislation was introduced (i) to impose inspection and control, and so prevent the sale of hydatid-infected offal from slaughterhouses or knackeries, or to ensure that sterilisation by thorough cooking (20 minutes at a rolling boil has recently been recommended) is carried out before such offal is released for sale to pet stores and (ii) to make hydatidosis a notifiable disease as suggested by the World Health Organisation.

Yours faithfully,

SHEILA WILLMOTT.

103 St. Peter's Street, St. Albans, Herts.

December 7th, 1959.

Enucleation of the Bovine Corpus Luteum

Sir,—I have been interested in the recent correspondence about the time at which the bovine corpus luteum may be enucleated without endangering the pregnancy, and I wonder if I might attempt to clarify the position?

The corpus luteum of pregnancy normally persists throughout the length of gestation in the cow (Hammond, 1927). McDonald, McNutt and Nichols (1953) found that pregnancy in 10 cows was terminated following the surgical removal of the corpus luteum between days 92 and 236. However, pregnancy was maintained in 3 cows that were 207, 224 and 230 days pregnant at the time of the operation. These results were confirmed and extended in a later study (Venable & McDonald, 1958), in which 1 animal aborted after removal of the corpus luteum on the 215th day, but 5 maintained their pregnancies when operated on between days 221 and 242.

Although one cannot exclude the possibility that the remaining ovarian tissue in these experiments was capable of producing sufficient progesterone to maintain the pregnancy, it seems more likely that there is some extra-ovarian source of the hormone. This is presumed to be the case in the human subject, horse, sheep, dog and cat, since in all these species the ovaries may be removed at some stage of pregnancy without producing an abortion. However, in the pig and the goat, ovariectomy at any stage of pregnancy will result in an abortion.

It is therefore interesting to speculate as to where this extra-ovarian progesterone could come from; by analogy with the human being and the mare, the placenta would seem to be the most likely source. However, a number of workers have failed to demonstrate any progesterone in cow placental extracts (Short, 1956; Pearlman, 1957; Gorski, Erb, Dickinson & Butler, 1958). On the other hand, Rakes, Hearn and Melampy (1958) claim to have isolated very large amounts of progesterone from this source; the concentrations that they found were so high as to cast some doubts on the validity of their method.

A possible alternative site of progesterone production is the maternal adrenal gland, and this has been shown to liberate relatively large amounts of

progesterone into the blood in cattle (Balfour, Comline & Short, 1957). At the present time, it is still not possible to evaluate the relative importance of these two extra-ovarian sources of progesterone in the cow.

One final point of some interest is the rate of progesterone production in the cow. The blood progesterone level seems to remain remarkably constant throughout pregnancy, although a sudden fall occurs just before calving (Short, 1958). Furthermore, the blood levels throughout pregnancy are probably no higher than those found in the luteal phase of the normal cycle. By analogy with the results in women and ewes (Short & Eton, 1959), it seems likely that the half-life of progesterone in the blood of cows is about 4 minutes. Using this fact, together with the concentration of the hormone in the peripheral blood, it is possible to arrive at an approximate estimate of the rate of progesterone production during pregnancy, as follows: —

Peripheral blood level of progesterone	= 1 μg. per 100 ml. plasma.
Total blood volume of a cow	= 40 litres.
Total plasma volume	= 20 litres.
Therefore, total amount of progesterone circulating	= 200 μg. (0.2 mg.).
Half-life of progesterone in the blood	= 4 minutes.

Therefore, every 4 minutes, 100 μg. must be produced to maintain a constant blood level, *i.e.* 36 mg. is produced every 24 hours.

This figure of 36 mg. per 24 hours is considerably lower than the maintenance dose of 100 mg. per 24 hours which McDonald found was necessary to maintain pregnancy in cows whose corpora lutea had been removed at an early stage of gestation (McDonald, Nichols & McNutt, 1952). The discrepancy between these two sets of results is probably due to the relative inefficiency of injections of a hormone that is metabolised so rapidly.

Yours faithfully,
R. V. SHORT.

ARC Unit of Reproductive Physiology & Biochemistry,
Department of Veterinary Clinical Studies,
School of Veterinary Medicine,
Madingley Road, Cambridge.

December 7th, 1959.

References

BALFOUR, W. E., COMLINE, R. S., & SHORT, R. V. (1957). *Nature, Lond.* **180.** 1,480.
GORSKI, J., ERB, R. E., DICKINSON, W., & BUTLER, H. (1958). *J. Dairy Sci.* **14.** 1,380.
HAMMOND, J. (1927). "Reproduction in the cow," Cambridge University Press, p. 127.
MCDONALD, L. E., MCNUTT, S. H., & NICHOLS, R. E. (1953). *Amer. J. vet. Res.* **14.** 539.
———, NICHOLS, R. E., & MCNUTT, S. H. (1952). *Ibid.* **13.** 446.
PEARLMAN, W. H. (1957). *Ciba Foundation Colloquia on Endocrinology.* **11.** 362.
RAKES, J. M., HEARN, W. R., & MELAMPY, R. M. (1958). *Iowa State Coll. J. Sci.* **33.** 13.
SHORT, R. V. (1956). *Nature, Lond.* **178.** 743.
———, (1958). *J. Endocrin.* **16.** 426.
———, & ETON, B. (1959). *Ibid.* **18.** 418.
VENABLE, J. H., & MCDONALD, L. E. (1958). *Amer. J. vet. Res.* **19.** 308.

Sir,—Dr. Y. Pincemin is to be congratulated on his very interesting series of observations in THE VETERINARY RECORD of November 21st, which constitutes an important contribution to knowledge in this sphere. At the same time the position is by no means so clear cut and indisputable as Dr. Pincemin supposes.

There is a considerable volume of literature on the subject; for brevity reference is made now to a few only of the more significant papers. Bretschneider, de Wit, and van der Kaay (1942) using a series of 73 pregnant cattle found that the corpus luteum increased in weight to a mean maximum of 5.2 gm. at 3½ months and thereafter fell away rather rapidly to 2.4 gm. at seven months and 1.8 gm. at term. The progesterone content and concentration graphs followed the same shape of curve and, histologically, there was a progressive replacement of granulosa cell by fatty tissue from the fourth to the seventh month. This first suggested that the corpus luteum might not be necessary in the second half of bovine pregnancy.

Following up this work, Raeside and Turner (1951) found that although exogenous progesterone was necessary to maintain a pregnancy when the corpus luteum was expressed from heifers in the early stages, a lower proportion of those heifers denied exogenous progesterone aborted, as the time chosen to express the corpus luteum advanced towards term. McDonald, Nichols and McNutt (1952) in a similar quantitative experiment on the supply of exogenous progesterone to maintain pregnancy after expulsion at the 60th day, found that abortion did not occur when progesterone injections were discontinued as early as the 162nd day, though it regularly did so up to that time. This was confirmed in a repeat experiment the following year. Yet Short (1958) showed that blood progesterone levels remained at a constant high from 32nd to 256th day, again suggesting that the hormone must in the later stages be produced elsewhere in the body (though not demonstrably in the placenta).

It is true that, as Dr. Pincemin appears to imply, sometimes an attempt to express a corpus luteum is quickly followed by the "reappearance" of the body (Clark, cited by McDonald *et al.*, 1953) and that only in a minority of the cattle (although frequently in pregnant sheep) used in the above experiments was this possibility eliminated by spaying. More work along these lines as well as along those ably pioneered by Dr. Pincemin is necessary to clarify the position. However, some may conclude that Mr. J. G. Wilson had some quite solid grounds for stating that expulsion of the corpus beyond midterm does not produce abortion, despite Dr. Pincemin's demonstration that it often does so in New Zealand. I am able to support Mr. Wilson, from observations here, that some pregnancies remain unaffected by corpus expulsion sometimes as early as at three months. In such cases there is no sign of any regenerated corpus at autopsy, which also seems to reveal a normal pregnancy.

The profession is also indebted to Mr. C. M. Gould for his observations on progesterone in

pyometra. It would be interesting to have more details about the anoestrous ovaries which Mr. Gould finds in the type of case which responds to this treatment. There is a suggestion in his letter of an association with cystic ovarian disease, in which event his good treatment results are in accord with theoretical expectations, since a review of the literature (Dawson, 1957) shows progesterone does appear to ameliorate cystic ovarian disease, in which pyometra is sometimes a secondary complication. In a study of 21 pyometra cases (Dawson, 1949) 4 associated with cystic ovarian disease were found, 10 with a retained corpus luteum and 6 with empty inactive ovaries; on general grounds one would expect only the disease type of the first 4 to benefit from progesterone treatment and that in the other 2 types the essential return to an ovarian cycle could not be achieved by exhibition of this hormone which would rather tend to prolong the anoestrus. These 21 cases comprised over one-fourth of a group of puerperal purulent endometritis cases studied intensively. As there is general agreement that the latter is a very common condition, perhaps Mr. Gould is fortunate to find pyometra a rare condition where he practises!

Yours faithfully,
F. L. M. DAWSON, A.V.I.O (Sterility).

Ministry of Agriculture, Fisheries and Food,
Veterinary Investigation Centre,
Madingley Road,
Cambridge.
December 4th, 1959.

References

BRETSCHNEIDER, L. H., DE WIT, D., & VAN DER KAAY, F. C. (1942). *Klin. Wschr.* **21**. 456.
DAWSON, F. L. M. (1949). *Diss. Cambridge University.*
———, (1957). *Brit. Vet. J.* **113**. 112.
MCDONALD, L. E., NICHOLS, R. E., & MCNUTT, S. H. (1952). *Amer. J. Vet. Res.* **13**. 446.
———, ———, & ———, (1953). *Ibid.* **14**. 385.
RAESIDE, J. I., & TURNER, C. W. (1951). *J. Anim. Sci.* **9**. 281.
SHORT, R. V. (1958). *J. Endocrin.* **16**. 426.

Sir,—The very title of the correspondence on this point is misleading as massage of the ovary has no effect on pregnancy whatsoever, and it would have been far better to state clearly that at least so far as termination of pregnancy is concerned in the intact animal the writers were referring to enucleation of the corpus luteum.

In my experience enucleation of this body in the first 3 months of pregnancy invariably results in abortion. If abortion does not follow then it is almost certain that either some residual luteal tissue was left behind during enucleation, or else that a second corpus luteum was present, perhaps in the other ovary, and that this was not removed. The examination of ovaries in living animals followed by the slaughter of the cows within a day or so can be a very humbling experience in this connexion.

During the last few years Dr. Moore (now of Sydney University) and I have been carrying out some experiments, part of which involved the transfer of ova in normal and above normal numbers to sheep which were ovariectomised at the time of transfer, *i.e.* 2 to 3 days after oestrus. Pregnancy was maintained by daily injections of either progesterone alone, in varying doses, or progesterone plus oestradiol benzoate. Despite the fact that these daily injections were all stopped at the sixtieth day of gestation the sheep went on to lamb normally at the expected time. In the sheep, therefore, the ovaries are not only unnecessary for the maintenance of pregnancy from the sixtieth day onwards, but they are also unessential for the induction of parturition and subsequent lactation.

Yours faithfully,
L. E. ROWSON,
Director.

The Gravel Pits,
Huntingdon,
Cambridge.
December 5th, 1959.

(Exotic) Operations on (Exotic) Reptiles

Sir,—After the recent correspondence in these columns concerning exotic operations I feel I must take this opportunity to mention a recent operation which I successfully performed.

A 4-year-old Indian spotted grass snake was brought into the surgery with the history of having swallowed a small ball-bearing; it showed considerable discomfort. On X-ray examination the ball-bearing was clearly shown up in the second part of the duodenum and an enterotomy was advised.

After the usual pre-operative procedure, a midline incision though the muscle was made and the ball-bearing was easily distinguished and removed.

The intestine was sewn up with 00000 gut using a Czerny-Lembert suture. The abundant ventral musculature was then approximated with similar gut, and the skin, which proved especially difficult, was sutured with fine nylon. 100,000 units of streptomycin were then administered intramuscularly and the diet was restricted for 3 days.

The snake made an uneventful recovery after a period of convalescence with a penicillin-enriched diet.

No literature was available on general anaesthetics which could have been used and the operation was performed under restraint and local anaesthetic.

Yours faithfully,
A. P. JONES.

23 Ravensbourne Gardens,
London, W.13.
December 6th, 1959.

The Use of Succinylcholine Chloride (Suxamethonium Chloride) for the Control and Management of Wild Animals

Sir,—In THE VETERINARY RECORD of April 18th, 1959, you kindly printed a letter by us on the use of Suxamethonium. On the basis of our early attempts to immobilise wild animals, we suggested that intramuscular injection of this drug might be useful for the control of horses that had become unmanageable on air or sea transport—as an alternative to destruction. We were, perhaps with justification, taken to task by Dr. Phyllis Croft who

pointed out several adverse aspects of the use of this drug.

It is not inconsistent with modern thinking to inflict a small amount of unavoidable pain on an animal to promote its own good. It is debatable if the fasciculations caused by Suxamethonium are as painful as, say, a small surgical procedure such as encasing a lower extremity of a dog in plaster of Paris to treat a simple fracture, and certainly it does not last as long. Furthermore, the more unpleasant side effects of the drug may be mitigated by addition of substances such as atropine, and work on these lines is in progress. We find that signs of fear may be greatly reduced in a wild animal by precautions such as walking quietly, covering its eyes, and talking in whispers and as little as possible. When those precautions are observed, animals appear to show no increased apprehension at the sight of humans and are not more difficult to approach than previously. Furthermore this drug is being used to save the lives of wild animals and, we hope, will save very many more in the near future. Animals of 9 different species have been successfully immobilised to date including rhinoceros, hippopotamus and several species of antelope, and we hope that we shall shortly have a considerable amount of information available to enable these methods to be extended to other territories.

To the British veterinarian the application of veterinarian science to wild animals outside captivity may not seen immediate. In Africa there is a growing body of opinion that wild animals, especially wild ungulates, are very necessary to the country's economy and that the present increasingly rapid destruction of these animals should cease, and the haphazard methods of eradication replaced by scientifically controlled cropping and utilisation. The main rôles in which wild animals are believed to be capable of playing an important part are as follows: (1) as a natural resource in proper land utilisation; (2) as part of the normal balance of soil flora and fauna and thus a factor in land conservation; (3) as a source of protein food that may yield more with a much smaller degree of destruction of land than that which can be obtained from domestic stock; (4) as an attraction for foreign capital. So far the only control of wild animals has been through destruction. Not only have they been shot for food and sport, but they have been shot wherever they came into conflict with agricultural pursuits, wherever they were thought to endanger human life, whenever an animal was sick or damaged, and in many cases whenever they crossed a game reserve boundary. They have been shot unselectively; the young and the female being killed as readily as the older male.

There are a number of species of animals in Africa in immediate danger of extinction, others are on the verge of a decimation that will virtually preclude their building up again into useful numbers. It is our object to save as many of those animals as possible through immobilisation and transportation as an alternative to shooting, and to assist the preservation of others mainly through increasing our knowledge of their migration cycles and movement patterns, as well as their food habits, dietary needs, etc. A few days ago we made a pilot experiment in immobilising a number of wild buffalo in a National Park and in vaccinating these animals against rinderpest, a disease to which they are highly susceptible. We hope that our experiments in marking animals such as hippopotamus in connexion with cropping schemes will assist in a proper estimation of the numbers that can be killed, and indicate the areas which the killing will affect. Pilot experiments have been completed on the moving of animals such as giraffe and kob antelope from areas bordering arable land to marginal areas where their survival may be permitted.

We feel that with the development of this technique of immobilising wild animals a weapon has been forged that is assisting in wild life preservation and may be instrumental in saving many more animals in the future. Through its use game management in its more immediate sense is becoming possible, thus adding an enormous section of interest to the veterinary field in countries such as Africa where large tracts of undeveloped and marginal land lend themselves to wild life utilisation and preservation.

Lastly, we would refer to the excellent article on the use of succinylcholine chloride by Mr. Neal and Professor Wright in your issue of October 17th, which has just come to hand after the draft of this letter was completed.

Yours faithfully,

J. A. LOCK,
A. M. HARTHOORN.
Departments of Medical Pharmacology, and Veterinary Physiology, Makerere College, Kampala, Uganda.

November 23rd, 1959.

DISEASES OF ANIMALS ACT, 1950—GREAT BRITAIN

Summary of Returns of Confirmed Outbreaks of Scheduled (Notifiable) Diseases

Period		Anthrax	Atrophic Rhinitis	Foot-and-Mouth	Fowl Pest	Sheep Scab	Swine Fever
1st to 15th Nov., 1959		16	—	—	251	—	75
Corresponding period in	1958	2	—	—	25	—	38
	1957	13	1	8	72	—	43
	1956	14	—	7	70	—	17
1st Jan. to 15th Nov., 1959		224	2	9	979	—	1,133
Corresponding period in	1958	139	5	116	642	—	1,107
	1957	290	8	125	816	—	852
	1956	1,197	11	135	673	—	667